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CONTENTS OF VOL. 38, NO. 4

ERRATA

Annals of Applied Biology, **36**, 2.

Page 193, line 19. *For* sentence beginning 'The analogy between...' *read*: The analogy between the new calculations and those for multiple regression leads to a simpler method of computing χ^2: in fact

$$\chi^2_{[3]} = S_{yy} + \frac{n_c(c-C)^2}{C(1-C)} - bS_{xy} - \frac{\delta C}{1-C} S_{ty} \qquad (12)$$

$$= 17{\cdot}00 + \frac{99 \times 0{\cdot}034^2}{0{\cdot}59 \times 0{\cdot}41} - 3{\cdot}795 \times 4{\cdot}282 - 0{\cdot}0137 \times 18{\cdot}693$$

$$= 0{\cdot}96.$$

Annals of Applied Biology, **37**, 4.

Index of Authors, page xi, lines 15 and 16, should read:

JOHNSON, C. G. Infestation of a bean field by *Aphis fabae* Scop. in relation to wind direction. (With Plate 13 and 3 Text-figures) 441

Annals of Applied Biology, **38**, 2.

Page 344, legends to Figs. 7*a* and 7*b*. Transpose titles to read:

Fig. 7*a*. No treatment.

Fig. 7*b*. Effect of mercury.

Annals of Applied Biology, **38**, 3.

Table, page 590, should read:

	Trap A	Trap B
Unidentifiable aphids	14	9
Haemerobiids identified and sexed	127	18

Annals of Applied Biology, **38**, 4.

Fig. 1, page 867, ordinate should read:

Mean yield per row of 10 plants.

THE SPREAD OF BEET YELLOWS AND BEET MOSAIC VIRUSES IN THE SUGAR-BEET ROOT CROP

I. FIELD OBSERVATIONS ON THE VIRUS DISEASES OF SUGAR BEET AND THEIR VECTORS *MYZUS PERSICAE* SULZ. AND *APHIS FABAE* KOCH

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(With 3 Text-figures)

A survey of aphids and virus diseases of sugar-beet root crops in eastern England was made between 1940 and 1948. Prior to 1943 the observations were made on fertilizer experiments; from 1943 onwards they were made on commercial fields selected for position in relation to beet and mangold seed crops. The incidence of beet yellows increased with increasing numbers of *Myzus persicae*, but not of *Aphis fabae*. The relation with *M. persicae* was sufficiently close to suggest that it is the most important, possibly the only important, vector of beet yellows virus. Beet mosaic virus also increased with increasing numbers of *M. persicae*, but the relation was not close enough to exclude the possibility of other vectors.

Numbers of *A. fabae* on sugar beet were slightly, but consistently, depressed by the use of salt as a fertilizer. Other fertilizers had variable effects. Neither aphids nor virus are likely to be greatly affected by fertilizers.

Beet yellows is most prevalent in areas where seed crops are grown, but within these areas nearness to individual seed crops did not appear to increase its incidence. *M. persicae* were more numerous on sugar beet in seed-crop areas than elsewhere, and this alone might account for the prevalence of yellows. Beet mosaic virus is more closely associated with seed crops than is beet yellows. It is most prevalent near to seed crops within the seed-crop areas.

INTRODUCTION

A series of observations on sugar-beet crops in the eastern and central counties of England was made between 1940 and 1948. The object was to relate the incidence of infection with beet yellows and beet mosaic viruses to the amount of infestation by aphids, and also, between 1943 and 1948, to the position of the observed fields in relation to beet and mangold seed crops.

Some of the results have already been given in articles and reports, and they have been used to provide information on which a possible means of controlling virus infection in the root crop could be based. The present control programme depends on the assumption that beet and mangold seed crops are the main sources of the viruses. These crops become infected in the autumn of their first year of growth, in what is known as the steckling stage. If they are the main sources of

infection, then preventing the viruses from reaching these stecklings, by isolation or protective measures, should go far towards preventing general outbreaks of virus in the root crops.

However, although root crops in seed-crop areas obviously suffer more frequent and more severe outbreaks of virus disease than crops in other areas, it has remained uncertain whether this is caused by the seed crops providing sources of infection, or by other factors which affect virus spread in the seed-crop areas. A detailed examination of the present data has been made, to define more fully the main factors concerned in virus distribution and to determine their relative importance. The methods used, and the general results of the survey, are described in the present paper. A detailed analysis and interpretation will be given later.

The data are not only of practical importance, but they also contribute to general knowledge of the epiphytology of virus diseases. Sugar beet has several advantages over other crops for this kind of investigation.

The viruses are not seed-transmitted, and the root crops start every year free from them. Symptoms usually become obvious in the field about 3–5 weeks after infection.

Sugar beet, mangolds and red beet are all sown during a period of about 2 months in the spring, and harvested during about 3 months in the autumn. With crops such as brassicae or legumes cropping of one variety or another is continuous throughout the year, and factors which influence the levels of infection, such as age of plants or nearness to the source of infection, are more variable.

So far as is known, beet yellows virus infects only species of Chenopodiaceae. Beet mosaic has hosts in other families, but they appear to be of little importance in Great Britain. Except for *Beta maritima*, which has a localized habitat, all the common susceptible chenopodiaceous weeds are annuals, so that these are not important sources of overwintering infection.

Sugar beet and mangold seed crops have a restricted distribution, whereas with most other crops the suspected sources of infection are as widespread as the crops themselves. The seed crops are not the only sources of infection, for clamped mangolds, and horticultural crops such as spinach or spinach beet, also contribute towards spread of infection. The influence of clamped mangolds has been studied by Broadbent, Cornford, Hull & Tinsley (1949). It has not been considered in the present investigation. Assuming that mangolds have a fairly even distribution throughout the country their effect on the results would merely be to increase the errors.

THE SCOPE OF THE SURVEY

Fig. 1 shows a map of the districts included in the survey with the observation fields marked by numbers. The years in which they were observed are given in the legend. The shaded parts show the areas with over 50, or between 10 and 50 acres of beet or mangold seed crop, in 24 sq. miles. The figures are derived from averages taken over 3 years, 1944, 1946 and 1947. Fields on the borders of the shaded region

were regarded as within seed-crop areas, although in some years they might not have been, because seed acreages in these areas varied from year to year. This only affected fields situated on the Lincolnshire side of the Northants-Lincs border, and some in the area south of Peterborough.

From 1940 to 1943 counts of aphids and virus diseases were made on fertilizer experiments carried out by the sugar factories in collaboration with Rothamsted Experimental Station. From 1943 onwards observations were made on commercial fields, chosen for their situation in relation to beet or mangold seed crops. Group O were outside and group S were within the seed area, as defined above. Group S was divided into two subgroups, S_1 and S_2, according to whether the fields were more than 1 mile, or less than 100 yd., from a seed crop. Originally group O had a subgroup of fields situated near to the small isolated seed crops which occur in most counties, even outside of the seed areas. But, especially in the later years, it was difficult to find such fields with the necessary requirements. There were too few to provide a reasonable measure of the influence of these seed crops on infection, so, for the purposes of this paper, they were discarded or included in the other groups.

Care was taken that the fields should resemble each other as much as possible except for their distance from seed crops. Particular attention was paid to sowing date and, as nearly as possible, all the observation crops were sown in the first half of April.

Observations made on the fields

At intervals of about 3 weeks, from June to September or October, the numbers of *Myzus persicae* (Sulz.) and *Aphis fabae* (Koch) present on the plants were counted. Alatae, adult apterae and nymphs were counted separately, but the figures used in this paper are for adult apterae only, because these were counted most accurately, and their use reduces the arithmetic variability between counts, as nymphs were usually several times as numerous as adults. The numbers of alatae were better determined from the sticky trap records which were kept from 1943 onwards.

From 1940 to 1942 only a few isolated specimens of *M. persicae* were seen on sugar-beet plants, but *A. fabae* was found regularly, sometimes in very large numbers. Therefore *A. fabae* was considered to be of primary importance, and *M. persicae* was largely ignored. At this time sugar beet had not been recorded in the literature as a regular host of *M. persicae*, and the aphid was not regarded as a pest of any economic importance in that crop. It was not until 1943 that it was found in sufficient numbers to suggest the part it is now known to play in the spread of beet virus diseases.

Other species of aphids were observed on the crops, but usually they were present only as winged migrants, and did not breed on sugar beet. *Macrosiphum euphorbiae* (Thomas) was found breeding on beet, occasionally in quite large numbers, but it occurred very erratically, and it has proved to be a poor vector of

the viruses in glasshouse tests, so has not been included in the present account of the survey.

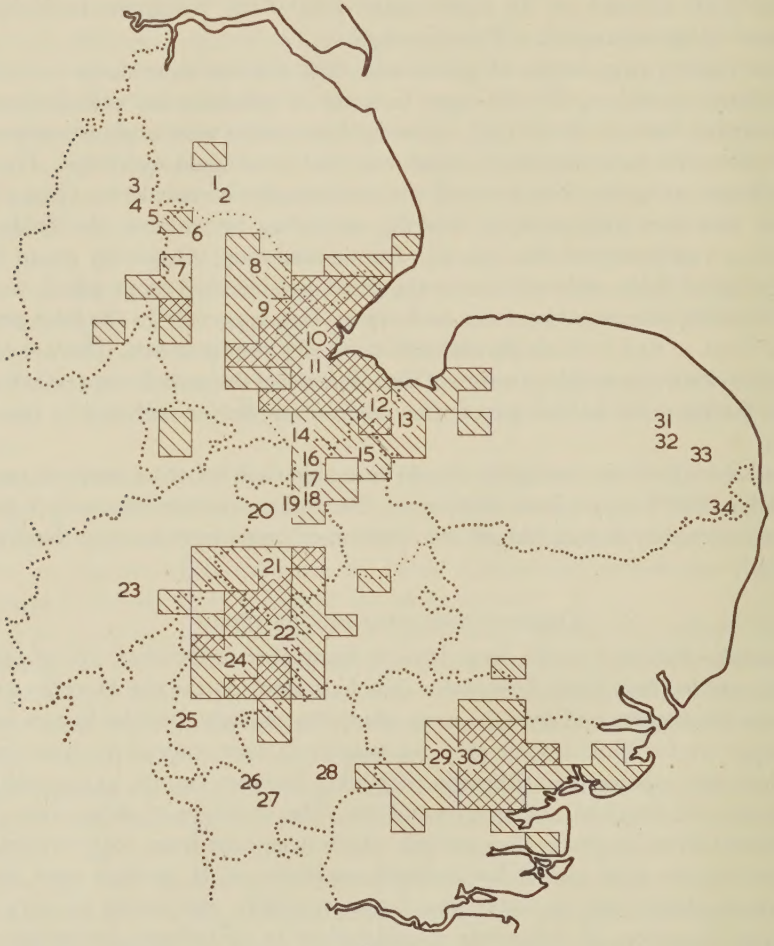

Fig. 1.

METHODS OF SAMPLING

A sample for aphid counting consisted of two neighbouring plants, examined *in situ* by turning over the leaves. If the aphids were numerous two half-plants were examined, and if very numerous one-fifth of the leaves on each plant was examined. The phyllotaxy of the sugar beet is such that a one-fifth sample of leaves of successive ages is easily selected by following diagonally up the crown from the oldest to the youngest leaf. Leaf numbers per plant were recorded so that aphid counts could be reduced to a leaf basis if required, but as the plant is the unit of

infection it seemed better to keep the aphid counts on a plant basis. The counts were expressed as aphids/100 plants to avoid fractions where there were fewer aphids than plants. Numbers of healthy and virus-infected plants were counted on the lengths of row between samples, and virus counts were expressed as percentage of counted plants showing symptoms.

The experiments observed up to 1943 had thirty-two plots carrying a factorial arrangement of fertilizer treatments. Samples were selected by random numbers, three from each of two rows on each plot. Thus 384 plants were examined at each observation time. Percentage infection was counted on rows taken at random to make up a count of about 150 plants/plot.

When whole fields were taken as units of observation the following method of sampling was used. Fields, or parts of fields, of from 5 to 10 acres were divided into four quadrants by counting the rows and pacing along the rows. These were permanently marked out by sighting posts. A random starting point was selected by one random number from half the numbers of rows in the quadrant, and another from one-sixth (later one-tenth) of the number of paces along the rows. The first sample was examined at this point. A traverse was then made systematically, the

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Legend to Fig. 1.

Fig. 1. Map of eastern England showing seed-crop areas and sites of observation fields.
..... County boundaries.
Area with 10–50 acres of beet or mangold seed crop in 24 sq. miles (6 × 4 miles).
Area with more than 50 acres of seed crop in 24 sq. miles.

Observation fields

Out of seed area

1 Hackthorn, 1944, 1945, 1946, 1947, 1948.
2 Welton, 1945, 1946 (Grange de Lings), 1948.
3 Rampton, 1943, 1944*, 1947.
4 Darlton, 1943, 1945, 1946.
5 South Clifton, 1945.
19 Farcet Fen, 1940, 1943; Blackbush, 1942.
20 Norman Cross, 1943.
23 Moulton, 1940, 1941, 1942, 1947.
25 Woburn, all years except 1942, 1943.
26 Rothamsted, all years.
27 St Albans, 1945, 1946.
28 Ware, 1941, 1942.
31 Coltishall, 1945.
32 Sprowston, 1945, 1947.
33 Burlingham, 1946.
34 Gillingham, 1946.

Within seed area

6 Harmston, 1944, 1946, 1947.
7 Brandon, 1944; Brant Broughton, 1946, 1947.
8 Blankney, 1943*; Digby, 1944.
9 Sleaford, 1943*.
10 Quadring or Quadring and Surfleet, 1944, 1945, 1946, 1947.
11 Spalding, 1945, 1946, 1947, 1948.
12 Wisbech, 1944, Tydd, 1945, 1946, 1947.
13 Terrington, 1947.
14 Postland, 1945, 1946, 1947, 1948.
15 Guyhirn, 1945, 1946.
16 Thorney, 1943, 1944.
17 Whittlesey, 1944.
18 Pondersbridge, 1943, 1944, 1945, 1946, 1947, 1948.
21 Buckden, 1944, 1945, 1946, 1947, 1948.
22 St Neots, 1944, 1945, 1946, 1947, 1948.
24 Elstow, 1947.
29 Dunmow, 1945, 1946, 1947.
30 Felstead, 1940, 1942, 1945, 1946, 1947.

* Two fields observed.

observer advancing one-sixth of the length of the row and one-sixth of the number of rows for each sample. Counts of infected and healthy plants were made on the row length between one sample and the next. If the edge of the quadrant was reached before the traverse was completed, it was continued from the opposite side of the quadrant. This method ensured that an objective sample was taken, and that every part of the quadrant had an equal chance of being selected for observation on any occasion. Analysis of variance was used on the data and showed that the sampling errors were small, and comparisons between fields and between occasions reasonably accurate. At first two traverses were made in each quadrant, but the analyses showed that there was no correlation between plants within traverses, and that effort could be saved without losing much accuracy by reducing the number of traverses, and increasing the number of observations to ten per traverse. This was done from 1944 onwards.

The row lengths counted for estimating percentage infection usually varied from about thirty to sixty plants, so that most of the figures are based on counts of about 1200–2400 plants/field.

In 1943 the sticky traps used for trapping winged aphids were painted 2 × 2 in. posts erected with their tops about 6 ft. from the ground. The top 3 ft. were painted with a proprietary grease-banding preparation. This was modified from the method used by Doncaster & Gregory (1948). Aphids were picked off the trap on the field, when the observation counts were made. Later, traps with removable covers were used (Broadbent, Doncaster, Hull & Watson, 1948), and these were changed weekly by the sugar-factory field staffs. The specimens were preserved in phenol, and identified during the winter. The species in which we were mainly interested were *M. persicae* and *A. fabae*.

The identification of alatae under a medium-power dissecting microscope was not without possibility of error. Two species, *M. certus* (Wlk.) and *M. caryophyllacearum* (n.sp. Lambers), are indistinguishable in the alate form from *M. persicae* (Hille Ris Lambers, 1946), and several *Aphis* species are difficult to distinguish from *A. fabae* (Jones, 1942, 1945). In view of the constancy with which the trap-count figures relate to the virus figures, it does not seem that the inclusion of other species than *M. persicae* could have introduced very large sources of error. The trapping of *A. fabae* did seem to be subject to error, but there was no indication that this was due to the inclusion of species other than *A. fabae* (see p. 753). Sugar beet is not a known host of any of the doubtful species, so that there was not the same difficulty in identification on the plants.

CHANGES IN APHID POPULATION THROUGHOUT THE SEASONS

(1) *Counts of aphids on the plants*

Table 1 gives the counts of adult apterae/100 plants of *M. persicae* and *A. fabae* made at intervals throughout the season in the years 1943–8. The figures for 1940–2 will be discussed later.

The observation periods, dates of which are given in the headings to Table 1, were adapted from the 8-day periods used by Doncaster & Gregory (1948). Each month is divided into four periods, the first three of 8 days each and the fourth containing the remaining days of the month. These periods fall into the same months each year, which calendar weeks do not. Each of the observation periods used in Table 1, and in later tables, contains three 8-day periods.

The actual dates of observation fell at different times within the observation periods and did not necessarily coincide with the culmination points of the stages of infestation concerned.

There seem to be five clearly marked stages in the development of an aphid infestation on the field, and it may be useful to define these so that they can be referred to briefly in the text. Stage 1, initial infestation, covers the period of entry of winged migrants into the crop. It usually overlaps with stage 2, development, which starts when the nymphs produced by the spring migrants become mature apterae, and continues until the emergence of alatae of the summer migration. Stage 3 is the peak infestation time, when aphid numbers become maximal and summer migrants leave the crop. Stage 4—decline, when very few or no aphids are found. Stage 5—autumn re-infestation, repeats stages 1, 2 and 3, usually on a smaller scale, in September or October.

The initial infestation usually occurred in May or June for *M. persicae* and in June or July for *A. fabae*. The first traces are indicated in Table 1 by the symbol 0*, which means that no adult apterae were found but that alatae and/or young nymphs were present. In 1946 and 1947 *A. fabae* appeared at about the same time as *Myzus persicae*.

As Doncaster & Gregory have pointed out, nymphs from migrant *M. persicae* were found in ones and twos usually without the parent insect, showing that the entering migrants moved frequently about the crop. Alate *A. fabae* were usually found with their offspring, often about ten per parent, showing that *A. fabae* moved less frequently than *M. persicae*.

The length of the development stage varied from about 3 to 8 weeks. Sometimes it might be said to continue indefinitely, especially with *A. fabae*, which sometimes bred slowly throughout the season. Sometimes there was no clear peak infestation and few migrants were produced. This was seen at several centres in 1946.

The time of initial infestation did not necessarily determine the time of the peak. The proportion of peaks reached in period 4 (the last 3 weeks of July) was 75 % for *M. persicae* and 70 % for *A. fabae*, but the initial infestations were about half in period 2 and half in period 3. Late peaks were more common with *A. fabae*, and early peaks with *M. persicae*. *M. persicae* had 20 % of its peaks before period 4, and only 5 % later than period 4, whereas *A. fabae* had 25 % later than period 4, and only 5 % earlier.

The decline stage of a normal infestation starts abruptly with a rapid decrease in the numbers of aphids. This is caused by the substitution of winged migrants

TABLE 1. *Seasonal and annual changes in numbers of adult apterae of* Myzus persicae *and* Aphis fabae *per* 100 *sugar-beet plants*

Observation periods: 2=25 May–16 June; 3=17 June–8 July; 4=9 July–31 July; 5=1 August–24 August; 6=25 August–16 September; 7=17 September–8 October; 8=9 October–31 October.
0*=alatae and/or young nymphs present but no apterae.

	Myzus persicae Observation periods						*Aphis fabae* Observation periods					
	2	3	4	5	6	7	2	3	4	5	6	7
1943, non-seed area:												
Rampton	—	52	0	0	0	0	—	0	25	26	2	0
Darlton	—	17	1	0	0	0	—	0	36	44	75	19
Norman Cross	—	26	6	0	0	0	—	0	2	0	0	0
Rothamsted	1	18	2	0	0	1	0	3	7	32	0	5
1943, seed area:												
Blankney	—	36	54	1	0	4	—	0	36	56	2	22
Blankney	—	24	68	4	2	2	—	0	24	110	20	4
Farcet	—	21	103	0	0	38	—	0	2	0	0	0
Sleaford	—	371	120	4	2	12	—	0	8	0	8	6
Sleaford	—	403	140	8	12	22	—	0	12	16	4	26
Pondersbridge	—	37	66	0	0	—	—	0	0*	0	0	—
Thorney	—	110	136	2	0	—	—	0	10	0	0	—
1944, non-seed area:												
Rampton	0*	2	15	—	0	0	8	25	1,088	—	0	0
Rampton	0	0	125	—	0	0	0	0	1,070	—	0	0
Hackthorn	0	5	1,260	18	0	14	5	5	668	151	0	17
Woburn	2	10	170	0	0	—	0	0	265	5	0	—
Rothamsted	—	2	80	0	0	—	—	0	1,090	0	0	—
1944, seed area:												
Harmston	—	8	214	45	—	0	—	0	454	5,392	—	0
Brandon	—	30	1,038	65	—	0	—	0	180	81	—	0
Digby	0	60	269	9	—	0	0	0*	94	894	—	0
Surfleet	6	12	1,120	0	0	0	0	1	1,560	1	0	0
Wisbech	18	105	1,210	0	0	0	0	0*	1,070	0	0	0
Thorney	2	24	1,338	0	0	0	0	0	1,888	0	0	0
Pondersbridge	0	26	782	0	0	3	0	0	255	0	8	10
Whittlesey	5	14	800	0	0	0	0	0	415	0	0	0
St Neots	1	38	220	0	0	0	0	0*	6162	0	0	0
Buckden	4	20	188	0	0	0	0	0*	2,052	0*	2	0
1945, non-seed area:												
St Albans	—	20	77	0	0	0	—	4	222	76	5	0
Rothamsted	—	84	0	—	0	0	—	62	20	—	0	0
Woburn	2	267	0	0	0	—	10	15	10	1	—	70
Sprowston	5	1,931	10,206	0	0	520	0	9,810	61,070	1	6	465
Coltishall	—	46	190	0	0	—	—	12	1,078	12	25	—
South Clifton	0*	31	437	7	0	94	0	0	12	2	0	0
Darlton	0*	10	130	1	0	0	0	0*	140	83	0	0
Hackthorn	0	14	49	4	0	0*	0	0*	31	345	0	50
Welton	0	36	128	15	0	90	0	0	70	105	5	172
1945, seed area:												
Pondersbridge	26	13,633	34,800	2	0	2,137	0	209	2,575	2	0	7,562
Postland	1	7	200	0	—	—	0	0*	497	117	—	—
Spalding	160	17	26	0	0	52	0	0	19	87	0	428
Quadring	3	464	9,562	2	0	802	0	0*	5,095	7	0	635
Tydd	—	5	92	2	—	0	—	0*	420	62	—	0
Guyhirn	9	71	130	8	0	30	0	0	50	0	0	25
St Neots	0	5	1	0	0	—	0	0	380	0	0	—
Buckden	12	45	10	0	0	0	0	7	82	5	0	15
Felstead	0	22	0	0	—	0	0*	10	2	0	—	0
Dunmow	5	95	0	0	—	0	0*	47	2	0	—	0

TABLE 1 (*continued*)

	Myzus persicae Observation periods						*Aphis fabae* Observation periods					
	2	3	4	5	6	7	2	3	4	5	6	7
1946, non-seed area:												
St Albans	0*	10	12	0	—	0	0*	63	95	12	—	0
Rothamsted	—	1	5	0	—	0	—	0*	120	0	—	0
Woburn	—	4	20	0	—	0	—	0*	3	12	—	10
Gillingham	0*	1	0*	1	0	0	0*	1	33	10	8	840
Burlingham	3	0	41	0	0	0	1	20	16	11	20	48
Hackthorn	0	0*	10	3	1	—	0	0	15	76	321	—
Welton	1	0	23	4	0	50	0*	0	54	54	254	98
Darlton	—	0*	28	8	0	—	—	0	98	58	21	—
1946, seed area:												
Pondersbridge	5	65	90	18	0	0	0*	0	37	0*	0	0
Postland	0*	11	393	20	0	48	1	1	13	120	0	121
Spalding	0*	18	53	3	0	1	0*	0	505	85	0	4
Surfleet	4	6	225	8	0	0	1	3	23	40	0	6
Tydd	0*	3	10	5	0	0	0	1	20	4,100	0	19
Guyhirn	0	9	225	3	0	40	0	0	115	155	15	168
Harmston	0	6	4	0	0		0*	0	33	175	158	—
Brant Broughton	0	6	6	5	0	—	0	5	80	371	69	—
St Neots	0*	3	0*	0	0	0	0*	1	225	5	0	10
Buckden	3	1	3	0	0	0	0	0	50	0	3	10
Felstead	—	1	48	0	—	0	—	0*	43	353	—	0
Dunmow	—	0*	62	0	—	0	—	0*	45	72	—	4
1947, non-seed area:												
Rothamsted	1	—	260	0*	—	2	9	—	750	0*	—	10
Woburn	—	57	—	0	0	10	—	87	—	0*	0	115
Sprowston	—	680	23	0	0	0	—	29,253	0	0	0	0
Rampton	—	0	2	2	0	0	—	1	1,060	10	0*	215
Hackthorn	—	0	40	5	0	0	—	0	292	32	75	0
Welton	—	—	10	17	2	0	—	—	87	422	0	0
Moulton	0	51	—	2	0	—	0	92	—	5	0	—
1947, seed area:												—
Pondersbridge	1	—	527	0	0	205	14	—	2,742	0	2	60
Postland	0	10	140	0	0	2	1	55	240	0	20	25
Spalding	0	307	10	0	—	52	0	80	2	0	—	0*
Quadring	0	—	86	0	0	55	0	—	176	0	0	15
Tydd	0	0	23	0	0	62	15	55	0*	0	0	17
Guyhirn	0	0	21	0	0	12	1	37	12	0	0	55
Harmston	0	—	507	15	0	177	27	—	807	45	0	212
Brant Broughton	—	2	0	0	2	—	—	275	592	5	0	—
St Neots	0	0*	—	17	2	12	1	25	—	15	42	65
Buckden	0	0	—	5	0	50	2	87	—	5	—	15
Felstead	—	0	20	0*	0	5	—	0*	40	0	0	42
Dunmow	—	10	280	0	0	40	—	50	35	0	0	790
Elstow	—	0*	107	0	—	32	—	9	120	5	—	357
1948, non-seed area:												
Rothamsted	—	0	65	—	—	—	—	0	235	—	—	—
Woburn	—	0*	105	0	—	—	—	0	187	0	—	—
Hackthorn	—	57	—	0	—	10	—	2	—	35	—	50
Welton	—	70	0*	—	0	2	—	0	0*	—	0	12
1948, seed area:												
Pondersbridge	—	—	73	—	—	0	—	—	36	—	—	0
Postland	—	—	12	—	—	0	—	—	77	—	—	0
Spalding	0	10	—	—	0	—	0	30	—	—	0	—
St Neots	0	—	—	0	—	0	0*	—	—	0*	—	0
Buckden	0	—	—	0	—	0	0	—	—	0	—	0

for apterae, but may be accelerated by parasites and predators. It is more complete for *M. persicae* than for *A. fabae*. Most sugar-beet crops are almost free from *M. persicae* for a time, between August and September, but a few *A. fabae* can usually be found. Large infestations usually ended suddenly; small ones were slower in developing, and, especially with *A. fabae*, tended to tail away more slowly.

The reasons for the change of the aphid population from apterae to alatae, and their avoidance of sugar beet as a host in the late summer, are obscure. Davidson (1929) showed that overcrowding induces the production of alatae, and that nutritional and other environmental factors also influence it. In addition, large numbers of aphids may attract parasites and predators which help to destroy the infestation. There is no question that parasites and predators do contribute to the decline, but whether they contribute proportionately more to the decline of large infestations than of small ones is open to doubt. The change in population composition cannot, in many cases, be due to overcrowding, because really overcrowded conditions in sugar beet are rare, especially with *M. persicae*. Well-defined peaks often occur when the populations are only 200 or 300 per 100 plants, far less than one aphid per leaf. It may be that as the plants grow older their food value to aphids decreases, and that this causes a change in population analogous to that caused by starvation induced by overcrowding. It is not the full explanation, for the plants always grow older at about the same rate, but the time of peak may vary from the middle of June to the middle of August. However, the time of peak is much more constant than the time of incidence. It does not depend on numbers of aphids present, or on the rate at which they have been produced, so it is likely that the nutritional value of the plants is important (see p. 755).

Reinfestation is not invariable on sugar beet. The autumn infestation was very small in 1944, although the peak period was early. In other years it varied from field to field. With *M. persicae* it is presumably initiated by migrants from other crops, since sugar beet is almost free from *M. persicae* during the decline period. *A. fabae* builds up on sugar beet during August and September in some years. The peaks of autumn infestation are usually small, and often the crops are harvested before they are reached, but they are important, because it is presumably these migrants which carry virus from the root crops to the stecklings. Stecklings are not above ground at the time of the summer migration, and were it not for the autumn migration, the problem of preventing virus infection of the following year's seed crop might be much simpler.

(2) *Counts of aphids on the traps*

Records were kept of about 60 aphid species caught on the sticky traps, but only the aphids presumed to be *M. persicae* and *A. fabae* are considered in the present investigation. There was a close correlation between the numbers of both *M. persicae* and *A. fabae* counted on the plants, and those caught on the sticky traps. The regression coefficient for the logs of the numbers of aphids/sq.ft. of trap on logs of

numbers/100 plants was $b = 1{\cdot}0493 \pm 0{\cdot}1342$ for *M. persicae*, and $b = 0{\cdot}432 \pm 0{\cdot}0885$ for *A. fabae*. Both are highly significant, but with *M. persicae* a ten-fold increase in field count corresponded with a ten-fold increase in trap count, whereas with *A. fabae* a ten-fold increase in field count corresponded with less than a three-fold increase in trap count. Either *A. fabae* produced only about a third as many migrants as *M. persicae* or the sticky traps were only one-third as successful in trapping them. This does not support the idea that aphids other than *A. fabae* were erroneously included in the sticky-trap counts, because this should have increased the proportion of trap-count to field-count aphids. Some results obtained by Broadbent (1948) may explain the disparity. He found that on sticky traps set at different heights, nearly two-thirds of the total count of *M. persicae* alatae were trapped at about 6 ft. from the ground, but the *A. fabae* complex of aphids were evenly distributed among traps set at 2, 4 and 6 ft. from the ground. Therefore our traps, set at 6 ft., would collect a larger proportion of the *M. persicae* than of the presumed *A. fabae* population.

Table 2 gives the numbers/sq.ft. of sticky trap of *M. persicae* and *A. fabae* averaged for group O and group S fields for eight observation periods (May to October) between 1943 and 1948. The average figures show clearly defined summer and autumn peaks, and the times correspond with those of peak infestation on the plants, so there is little doubt that the migrants trapped at these times are mostly from fields on or near which the traps were placed. Doncaster & Gregory refer to a 'spring peak' in their trap-count figures, coinciding with the time at which the migrants leave their winter hosts. This is ill defined in the sugar-beet figures, particularly in the seed-crop areas. Isolated specimens of *M. persicae* and *A. fabae* were caught for some weeks before and around the time of initial infestation, but, with a few exceptions, even on fields situated close together, these were very irregularly spaced in time, which explains their failure to appear as peaks in the average figures. The appearance of the average figures suggests that they were random catches from a small but gradually increasing population. This is in agreement with Broadbent & Doncaster (1949); they found that the proportion of the total catch of alate *M. persicae* and *A. fabae* trapped in May was smaller than in June or July.

There were, however, areas in which definite spring migration peaks of *M. persicae* were observed. These were at Rothamsted, at the Herts Farm Institute, St Albans, and at Woburn, Beds. In one year, 1948, there was a spring peak at Sprowston, near Norwich, and in one year a small peak at Spalding. A field near Milford, in Surrey, had 36 *M. persicae* per sq.ft. trap in early June 1947, and 64 per sq.ft. in May 1948. These fields were all near towns, or market-garden areas, or both, and were mostly in the southern counties. This suggests that most of the spring peaks occurred where there were plenty of overwintering vegetable hosts, and where rather mild weather conditions prevailed. In other areas the movement of spring migrants into the sugar-beet crops seems to have been more erratic.

TABLE 2. *Seasonal and annual changes in average numbers of alatae of* Myzus persicae *and* Aphis fabae *per sq.ft. of sticky trap*

O = outside of seed-crop area; S = within seed-crop area.

		Observation periods							
		1 1. v.–24. v.	2 25. v.–16. vi.	3 17. vi.–8. vii.	4 9. vii.–31. vii.	5 1. viii.–24. viii.	6 25. viii.–16. ix.	7 17. ix.–8. x.	8 9. x.–31. x.
					Myzus persicae				
1943	O	0·5	0·5	1·2	2·0	4·0	1·9	12·0	—
	S	—	0·0	0·3	12·1	7·7	0·6	2·1	—
1944	O	1·0	0·5	2·3	14·4	3·8	2·4	0·5	0·3
	S	—	0·6	1·0	70·1	12·2	0·1	0·0	—
1945	O	0·2	0·2	5·8	49·1	5·9	0·2	0·9	2·1
	S	0·1	0·0	9·2	263·0	9·4	0·5	0·6	3·5
1946	O	1·5	2·0	2·5	2·0	0·9	0·9	1·6	3·0
	S	1·0	4·1	4·3	7·8	10·0	0·4	1·0	2·0
1947	O	0·0	0·0	1·6	17·5	10·2	4·3	4·2	41·0
	S	0·1	0·3	1·0	26·8	9·2	1·5	4·1	23·3
1948	O	—	6·8	6·0	12·2	1·0	0·0	0·0	—
	S	—	3·7	15·2	9·0	4·8	0·2	0·0	—
					Aphis fabae				
1943	O	—	0·0	0·2	3·2	3·2	0·7	0·0	—
	S	—	0·0	0·0	1·7	1·5	0·3	0·8	—
1944	O	—	0·8	0·0	24·5	95·0	0·3	0·0	—
	S	—	0·0	0·1	15·6	30·2	0·2	1·0	—
1945	O	0·0	0·5	5·1	23·1	6·2	0·3	0·2	3·5
	S	0·1	0·1	1·3	6·0	0·6	0·4	2·0	3·3
1946	O	0·4	1·8	4·2	6·2	0·9	0·3	1·0	1·5
	S	0·4	1·0	1·9	5·5	7·9	0·7	1·3	1·7
1947	O	0·0	1·0	14·5	68·2	62·1	0·6	0·1	2·1
	S	0·1	1·3	2·4	124·0	29·1	0·3	1·0	1·9
1948	O	—	0·0	0·0	2·0	4·0	1·0	0·0	—
	S	—	1·0	1·8	2·4	1·2	0·0	0·4	—

Although there are so few *M. persicae* on the plants for a period in August or September, some alatae could usually be found on the sticky traps, which suggests that winged aphids were flying about the fields, but failing to colonize the sugar beet. Potatoes (Doncaster & Gregory, 1948) were never so free of *M. persicae* as were sugar beet, and some colonies could usually be found.

Kennedy, Ibbotson & Booth (1950) described feeding experiments with *M. persicae* and *A. fabae* in which the aphids were found to infest freely only the growing (young) and senescent (old) and not the mature (medium aged) leaves of sugar beet. Our results suggest that the aphids breed freely only on young or old plants, and not on those of medium age. It is possible that this behaviour is determined by nutritional changes of the same nature as those which cause differences within the leaves of a single plant. The observation (Kennedy *et al.* 1950) that *M. persicae* 'more strictly avoided the more mature (i.e. medium aged) leaves' of sugar beet than did *A. fabae* agrees with our observation that *A. fabae* continues to breed on sugar beet from which *M. persicae* has disappeared. These authors also suggest that the range of leaves on which an aphid feeds is a measure of its adaptation to the host plant. Thus *M. persicae* will infest a wider range of leaves on a potato than on a sugar-beet plant; this again agrees with the behaviour of the aphid in infesting the crops as a whole.

Observations on the fertilizer experiments

Table 3 shows the peak numbers of adult apterous *A. fabae* per 100 plants on thirteen experiments made between 1940 and 1943 to compare the effects of sulphate of ammonia, sulphate of potash, superphosphate, and agricultural salt, on the growth of sugar beet. The fertilizer experiments were discontinued at Woburn in 1941 and Rothamsted in 1942. Aphids and virus-infected plants were counted in other experiments, and the means for these are given to maintain continuity.

Details of the experiments are given in the Rothamsted *Annual Reports* for 1943–5. The treatments were factorially arranged and the fertilizers used according to ordinary agricultural practice. The mean numbers of *A. fabae* per plot are given in column 2 of the table, and the increases caused by the fertilizers are shown in the next four columns, followed by the standard errors of the fertilizer differences. The standard errors of the means can be obtained by halving the standard errors of the differences.

The effects of the fertilizers on the percentage of plants infected with yellows virus in September have already been described (Hull & Watson, 1948); the means for the thirty-two plots, with their errors, are given in the last column of Table 2.

The aphid populations differed widely in different years, but were reasonably constant in any one year. In 1940 the peaks were reached in late July or early August. For a short time there were very large numbers of aphids on the plants, but the infestations were destroyed by syrphid attack. In some fields, counted later

in August, the leaves were smothered with the dead bodies of aphids cast aside by the syrphid larvae. In this year the syrphids seemed to have prematurely reduced the infestation which was rather late in reaching its peak, but the condition has not been observed to the same extent in any subsequent year.

In 1941 aphids were fewer and the infestations developed slowly. In all four experiments, the populations increased throughout the season, and peaks were reached in middle and late September. The average count of adult apterae for late July was only 5, in August it was 200, and in September 312 per 100 plants.

TABLE 3. *The effect of fertilizer treatments applied to the crop on peak numbers of* Aphis fabae *apterae per* 100 *plants*

Site and year of exp.	Date of observation	Mean	Increase for fertilizers			S.E. of fertilizer effects	Percentage infection virus yellows in September
			P	K	S		
1940: Rothamsted	26. vii.	15034	− 18	1845	3568	−3395	±3111	10·3±2·6
Woburn	16. viii.	2731	−3056	− 350	−1019	−1488	± 790	3·3±0·5
Moulton*	25. viii.	178	− 161	30	− 39	− 151	± 69	4·6±0·6
Felstead*	30. viii.	1214	199	124	− 218	− 180	± 135	2·9±0·7
Mean	1940	4789						
1941: Rothamsted	28. viii.	471	129	142	20	− 326	± 101	2·2±0·6
Moulton	18. viii.	130	119	44	4	− 69	± 138	2·2±0·3
Hertford	4. viii.	169	62	− 25	25	− 138	± 49	1·7±0·2
Woburn	6. viii.	30	—	—	—	—	—	1·2 —
Mean	1941	200						
1942: Hertford	1. viii.	1280	− 641	− 307	− 931	−1346	± 693	8·0±1·1
Moulton	6. viii.	2262	1071	780	− 38	−1162	± 793	0·8 —
Blackbush	7. viii.	373	102	58	163	− 151	± 115	0·1 —
Rothamsted	15. vii.	1875	—	—	—	—	—	3·2±0·7
Mean	1942	1697						
1943: Bardney	16. viii.	193	75	− 12	− 10	− 118	± 77	33·0±3·9
Brigg	30. vii.	233	− 185	0	− 90	− 119	± 79	8·9±1·8
Newark	10. viii.	308	− 74	− 13	− 14	− 74	± 109	16·3±1·8
Mean	1943	245						

* Very many aphids killed by syrphids.

In 1942 numbers of *A. fabae* increased again; high peaks were reached in late July or early August. Few aphids were found in late August and September, and there was no autumn reinfestation on the observed fields.

Salt was the only fertilizer which had a consistent effect upon the numbers of *A. fabae*. On the average it depressed the populations by about 30 %. The results published previously show that the average effect of salt on yellows infection was a reduction from an average of about 17 % to about 14 %. This result was significant when calculated from the means of the log analyses of the original data, but it is small and unlikely to be of economic importance. As the effects of salt on *A. fabae*

and on infection are positively correlated, this may indicate that for the period 1940–3, and on the fields examined, none of which was well within the seed crop area, *A. fabae* was responsible for a small amount of virus spread. This is supported by the fact that, for this period only, the small annual fluctuations in percentage yellows infection also agreed with the large ones of *A. fabae*. Even over this period, however, yellows virus may have been spread by the very small numbers of *M. persicae*, and *M. persicae* may be affected by salt in a similar manner to *A. fabae*. There is no *a priori* reason why *A. fabae* should not affect spread of yellows, for glasshouse tests (Watson, 1946) show it to be a reasonably good vector, but the results of the survey have given no indisputable evidence that it does so.

The main deduction to be drawn from the results of aphid counts made on the fertilizer experiments is that the kind of variation in nutrient status found on ordinary agricultural crops is unlikely to introduce important variations in the incidence of virus diseases.

Variation in aphid populations between years

Table 4 gives the average counts of adult apterae per 100 plants for *M. persicae* and *A. fabae*, as totals of one count per observation period up to the end of August. The main field groups, O and S, and the years 1940–8, are given separately.

There were no counts of *M. persicae* for 1940 and 1941, although a few specimens were recorded. Doncaster & Gregory (1948) also record few *M. persicae* on potato crops in these years, except in Lincolnshire. In the Spalding area there were moderate numbers in 1940, and a moderately large autumn reinfestation in 1941. In other areas there appear to have been very few. Counts were not made on sugar-beet fields in the Lincolnshire area until 1943, and on the fields which were observed it can reasonably be assumed that, though some *M. persicae* may have been overlooked, there were very few compared with later years. In 1942, five *M. persicae* were recorded in one experiment, one and two respectively in two others. This shows that *M. persicae* were at least present, although the records may be inadequate.

Both the arithmetic and the geometric means of the counts are given, because the geometric means give less weight to exceptionally high populations, which otherwise tend to give a false impression of the distribution between the fields. When the geometric and arithmetic means differ widely, the variation between fields is great, and when they resemble each other, it is small. For instance, Table 1 shows that variation between fields was much greater in 1945 than in 1944 for both *M. persicae* and *A. fabae*. This is indicated in Table 4 by the relative differences between the arithmetic and the geometric means, and it is the main reason why logarithms have been used in Text-figures 2 and 3, and in later calculations. Counts of *M. persicae* and *A. fabae* in 1944 and 1945 varied similarly, but in 1947 there were some very high *A. fabae* counts, whereas the *M. persicae* counts were uniformly low.

It seems, therefore, that though there are factors which tend to control the levels of both species of aphids in all districts in any year, there are also individual causes of variation which can affect fields or districts, and sometimes the two aphid species, independently.

Of the many interrelated factors which determine the levels of summer infestation with *M. persicae*, one of the most important is winter temperature, which affects the survival of viviparous parthenogenetic forms on winter brassicae and other hosts

TABLE 4. *Average annual counts of* Myzus persicae *and* Aphis fabae *adult apterae per* 100 *plants for fields in and out of seed-crop areas. Totals of counts made up to the end of August*

	Arithmetic means			Geometric means		
	Non-seed area	Seed area	Mean of all counts	Non-seed area	Seed area	Mean of all counts
			Myzus persicae			
1940	0	—	—	0	—	—
1941	0	—	—	0	—	—
1942	2·3	—	—	1·7	—	—
1943	51	244	164	40	186	98
1944	338	760	619	132	600	398
1945	1505	5910	3824	245	211	226
1946	19	118	73	10	48	26
1947	131	115	122	38	25	30
1948*	24	23	22	13	13	13
Mean of all counts 1943–8	404	1273	901	46	124	81
			Aphis fabae			
1940	5122	—	—	2511	—	—
1941	144	—	—	89	—	—
1942	1592	—	—	1349	—	—
1943	88	40	55	39	15	19
1944	1292	1974	1402	968	1040	1941
1945	2039	969	1476	221	232	227
1946	235	556	411	178	170	174
1947	4159	1858	2730	729	203	326
1948*	94	36	58	50	29	36
Mean of all counts 1943–8	1642	1074	1300	256	166	197

* Fewer observations were made in 1948, so these figures are probably an underestimate.

(Jacob, 1941). At Rothamsted the years 1940–2 had an average of nearly 40 days of frost in January and February, and very few *M. persicae* were found in the following years. From 1943 to 1945 the average days of frost were only 27, and *M. persicae* increased greatly during that period. In 1945, although there were large numbers of spring migrants, a wet spring and, apparently, varying local conditions affected the development of the colonies so that the figures were more variable than in the previous year. Comparatively mild winter weather was also experienced in 1945–6, but cold, wet weather in June and July prevented the infestations from developing.

The prolonged frost and snow in the early months of 1947 greatly reduced and delayed the spring migration, both of *M. persicae* and *A. fabae*, but the exceptionally fine weather in June and July permitted large infestations, especially of *A. fabae*, to develop in some places. Many spring migrants of *M. persicae* come from winter vegetable hosts after a mild winter, whereas all those of *A. fabae* come from colonies established on spindle trees after hatching of the winter eggs. Therefore *A. fabae* numbers are less likely to be affected by low winter temperatures than those of *M. persicae*, but they are more likely to be critically affected by the spring weather, which determines the time of hatching and the development of colonies on spindle.

It has been mentioned previously that the age of the crop may affect the behaviour of the aphids at peak time and during the autumn reinfestation. It also affects the growth of the aphids during the development stage. In experiments in which varying sowing date has been used as a treatment, the younger plants have been found to develop the largest populations. For example, in an experiment made in 1941, to compare times of infection with beet yellows virus on plants of different ages, the following average numbers of *A. fabae* apterae were counted on 17 July: beet sown on 8 April, 51 aphids per 100 plants; beet sown on 5 May, 594 aphids per 100 plants; beet sown on 24 May, 1318 aphids per 100 plants; S.E. (13 D.F.) $\pm 37{\cdot}44$. Occasionally sowing date affects aphid numbers in the opposite way, but this is when the later sowings are so late as to miss the spring migration altogether, and so escape infestation.

Variation in aphid population between seed and non-seed areas

Table 4 shows that *M. persicae* was considerably more numerous in the seed-crop than in other areas, whereas *A. fabae* seemed to be rather more plentiful in the other areas. This difference seems most likely to be attributable to variations in the sources of aphids, which suggests that there are more sources of *M. persicae* in the eastern counties than in southern and central England, and vice versa for *A. fabae*. There may be more spindle trees in well-wooded counties than in the open fens of Lincolnshire, but it is not true that there are more sources of *M. persicae* in Lincolnshire than elsewhere, for large spring migrations of *M. persicae* mostly occur outside the seed areas. The difference presumably lies in the build-up of population on the crop when the aphids have entered it. The only factor which suggests itself as being likely to affect this in the observed manner is rainfall. High humidity or the cool temperatures associated with rainy periods probably depress aphid growth, and in sugar-beet crops heavy rain can reduce numbers very considerably. Thus it appears that the climate which makes the eastern counties suitable for seed growing also renders them more susceptible to outbreaks of virus diseases.

Although rainfall is less in the eastern counties, the open fenlands are usually very windy, and this, according to Davies (1936) and later workers, should deter aphid movement. However, Broadbent (1946) has shown that aphids will fly

in windy weather, presumably because the wind is reduced near the ground and in the shelter of the plants. In such conditions aphids might fly but not rise far above the plants unless they were caught by a gust of strong wind and carried away. This situation might favour spread of virus diseases within a crop. The smaller and more erratic spring migrations in the seed-crop areas may result from there being fewer and shorter periods of weather suitable for large migrations.

VIRUS INFECTION IN RELATION TO APHID INFESTATION

Fig. 2 shows the mean annual levels of percentage infection with beet yellows virus, counted on the observation fields in the third weeks of September, and also the average numbers of adult apterae per 100 plants of *M. persicae* and *A. fabae*. The aphid counts are totals of one count per observation period up to the end of August, and are given as means of logs. The figures for 1948 have been omitted because the counts of aphids on the plants were inadequate.

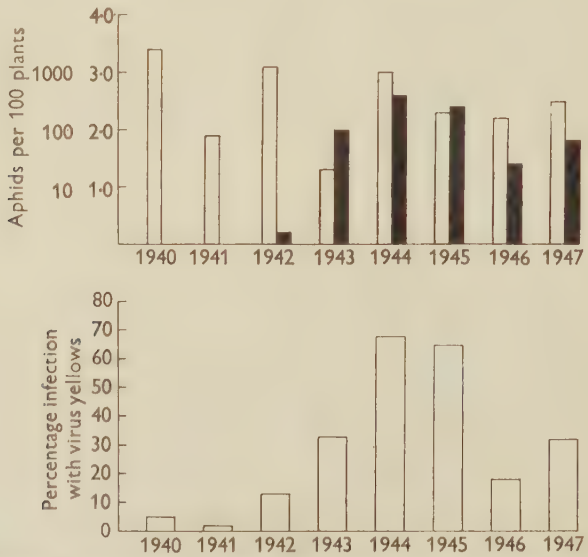

Fig. 2. Lower figure: average percentage infection with beet yellows virus. Upper figure: numbers of *Myzus persicae* and *Aphis fabae* for the years 1940–7. The aphid counts are given as means of the logs for total counts throughout the season for apterae per 100 plants. □ *A. fabae*; ■ *M. persicae*.

From 1940 to 1943, the numbers of *A. fabae* fluctuated widely, but there was little change in the level of infection with beet yellows virus, which remained low. In 1943 appreciable numbers of *M. persicae* were first found on sugar beet, and the level of yellows infection increased. Numbers of *M. persicae* and levels of virus infection remained high in 1944 and 1945. In 1946 *M. persicae* decreased, whereas

A. fabae were about as numerous as in 1945. However, the level of infection with yellows virus again dropped to a low level. These figures suggest strongly that yellows virus is mostly spread by *M. persicae*, and that *A. fabae* spreads very little. Doncaster & Gregory (1948) similarly record that they could detect a relationship in numbers of *M. persicae* and levels of infection with rugose mosaic and leaf roll of potatoes, but none with *A. rhamni*, although *A. rhamni* was often present in much larger numbers than *M. persicae*. They suggest that the more sedentary habit of *A. rhamni* is responsible for its unimportance as a vector. The departure of *A. rhamni* from a theoretical random distribution on the plants was much greater than that of

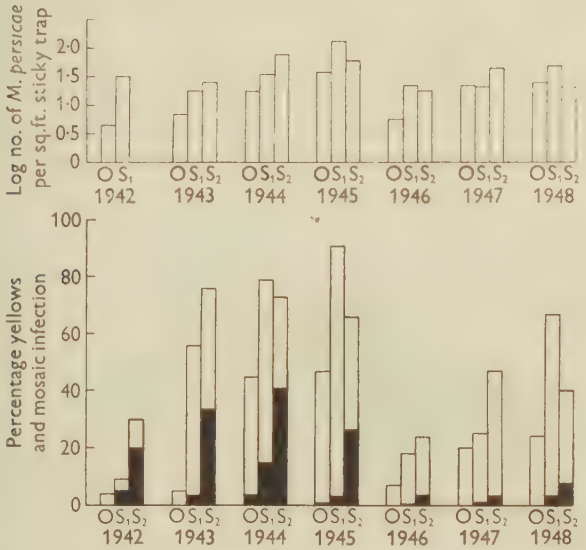

Fig. 3. Lower figure: average percentage infection with beet yellows virus (full height of column) and beet mosaic virus (black portion). Upper figure: average numbers of alate *Myzus persicae* per sq.ft. of sticky trap, given as means of logs of totals up to the end of August. O, fields out of seed-crop areas; S_1, within seed-crop areas but not near seed crops; S_2, within seed-crop areas and near to seed crops. □ Yellows; ■ Mosaic.

M. persicae. *A. fabae* on sugar beet behaves very like *A. rhamni* on potatoes. It is usually very irregularly distributed; in heavy infestations some plants may be smothered while others nearby are comparatively free. In all probability the failure of *A. rhamni* and *A. fabae* as vectors of viruses is caused by their lack of activity. Kennedy (1950) pointed out that, as transmission of virus depends on movement of the vectors, the less well-adapted pests are more likely to be good vectors than those better adapted to their host plants.

Fig. 3 shows the average annual levels of beet yellows and beet mosaic viruses compared with mean log counts of alate *M. persicae* per sq.ft. of sticky trap for fields grouped according to their situation in relation to beet and mangold seed

crops. In each histogram the first column represents fields outside the seed-crop area, and the second and third columns respectively give the data for fields far from, and near to, seed crops within the seed-crop areas.

The data for 1942 include some trap records published by Doncaster & Gregory (1948), which were obtained from potato fields near to those on which beet virus diseases were counted. Virus counts from a general survey of Lincolnshire and Huntingdonshire, made in September 1942, have also been used, because regular observations were made only on fields outside the seed-crop areas. The use of these data in a general table is justified, because the statistical results showed that most important relationships between virus and vectors can be determined from disease counts made in September and the sticky-trap figures.

The relation between level of infection with yellows virus and infestation by *M. persicae* is even more striking using the sticky-trap records than using the field-count records. It is apparent not only between years, but also between groups within years. Only in two instances do the aphid and virus counts vary in opposite directions.

There appear to have been two periods of increasing incidence of virus diseases during the period observed. The first was between 1942 and 1945, the second between 1945 and 1949. The present series of field observations finished in 1948, but a very serious outbreak of yellows in 1949 was recorded by the factory agricultural staffs.

In 1942 there was very little yellows in fields outside the seed-crop areas but much within them. In 1943 this relationship between seed and non-seed areas was maintained, but in 1944 the disease was much more widespread, and remained so in 1945. This seems to suggest that infection came from the seed-crop areas and spread to others, but it must not be ignored that the numbers of *M. persicae* were distributed between the different areas in the same way as the levels of infection. Numbers of *M. persicae* in the non-seed areas trebled between 1943 and 1944, and doubled again in 1945. If increase of virus infection were directly proportional to aphid numbers, this alone would account for the increase of virus in the non-seed areas. Further examination of the data has shown that the relationship is not linear, and that the differences in levels of infection cannot wholly be accounted for by differences in aphid numbers, but the direct comparisons do not indicate this. The question is of vital importance to the success of any control programme based on elimination of virus from the seed crops.

Apparently only the cold wet early summer of 1946 prevented a virus outbreak similar to that of 1945. Whatever the reason, the necessary level of *M. persicae* infestation failed to develop, and the level of infection dropped to about that of 1942. During the next two years it increased again in all areas, but more in the seed than in the non-seed areas, culminating in the very severe and widespread outbreak of 1949.

The distance from a seed crop within the seed-crop area seemed to have little or

no effect on level of infection with yellows virus. However, with beet mosaic virus the distance from the nearest seed crop had a pronounced effect upon prevalence of infection. Mosaic, like yellows, was most prevalent during 1943–5, but unlike yellows it never spread to the non-seed areas, and within the seed areas it was almost confined to fields which were within 100 yards of a seed crop.

This behaviour is readily explicable if the seed crops are accepted as the main sources of infection for both viruses. Beet mosaic is a non-persistent virus (Watson, 1946); it is picked up by the vectors during a short feeding time on the infected plants, but it is also lost very rapidly by the vectors, particularly if they are feeding. Beet yellows is a persistent virus; the aphids need a longer feeding time to acquire infectivity, and they may remain infective for several days. Therefore beet yellows has a much greater chance of being carried long distances from the source of infection than has beet mosaic virus. If this is the true explanation, it is strong evidence that the seed crops greatly influence the initiation of virus outbreaks, for all alternative hosts from which beet yellows virus could be obtained are equally susceptible to beet mosaic virus, and if yellows is regularly dispersed from these sources there seems no reason why they should not also act as sources of mosaic. The fact that mosaic is so closely associated with the seed crop, that there are good reasons why it should not readily be transmitted great distances, and that these reasons do not apply to beet yellows virus, provides strong circumstantial evidence that the seed crops are the main reservoirs of both viruses.

The increase of beet mosaic virus during the years 1943–5 was comparable with that of beet yellows virus, and both seemed to be associated with increase in numbers of *M. persicae*. From 1946 to 1948, however, the increase of yellows virus was more or less commensurate with the increase in numbers of *M. persicae*, but that of beet mosaic virus was not. This suggests that perhaps the association of beet mosaic with *M. persicae* as a vector is not so simple, or so complete, as that of beet yellows virus.

The authors wish to thank the numerous people who have helped with this survey from time to time, especially Dr F. M. Roberts, Dr W. M. Dion, Dr C. E. Cornford and Mr T. W. Tinsley of the Plant Pathology Department, Rothamsted; also the agricultural staffs of the British Sugar Corporation Factories, Norfolk Agricultural Station, Woburn Experimental Farm, Herts Farm Institute, Moulton Farm Institute and Mr W. A. Scriven of the Cambs (Isle of Ely) A.E.C. for help in finding fields and changing aphid traps. We also thank the many farmers who have permitted us to use their crops for observation.

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THE REACTION OF VIRUS-INFECTED POTATO PLANTS TO *PHYTOPHTHORA INFESTANS*

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(With 1 Text-figure)

The growth of *Phytophthora infestans* was retarded on leaves of potato plants that had been artificially inoculated with virus *X* or with virus *Y*.

Using different virus strains and potato varieties, the effect of virus infection on blight development was found to be greater, the more severe the systemic virus symptoms exhibited on the infected leaves before *P. infestans* inoculation.

The development of the fungus was never increased by virus infection.

The reduced blight development on virus-infected leaves is partially caused by an increase of resistance to infection. It is also suggested that virus infection alters the nutritional status of leaves to one less favourable for the development of *P. infestans*.

Observations on field crops of potatoes suggested that potato blight developed less rapidly when the plants were virus-infected than when they were not. This could have two explanations: changes in the size of leaves and size of plants by virus infection might alter the microclimate to one less favourable for the fungus; or the presence of viruses in the plants might influence their susceptibility to *Phytophthora infestans*. The second seemed more probable to us, and experiments were therefore undertaken to test it.

Materials and methods

Viruses and potato varieties

The varieties of potatoes and viruses used were President, British Queen, Craigs Alliance, King Edward, Arran Victory and Katahdin inoculated with strains of potato virus *Y*, and Majestic inoculated with strains of virus *X*.

All the potato plants used for the 'first year of infection' (primary symptoms) experiments were virus-free, uniform in size, age and vigour and about 5 in. high when they were inoculated each with a strain of virus *Y*. Several plants of each of the following varieties were inoculated with *Y* strains *Y*5 and *Y*6 (these and other strains of virus *Y* are to be described by Munro in a subsequent paper), President, Katahdin, Arran Victory and King Edward. A number of King Edward plants were also inoculated with strain *Y*3. The plants of varieties British Queen, Craigs Alliance, President and Majestic used in the 'second year of infection' (secondary symptoms) experiments were grown from tubers of those varieties inoculated the previous year with strains of viruses *X* and *Y*, as follows: British Queen *Y*7, *Y*8 and *Y*12; Craigs Alliance *Y*1, *Y*7 and *Y*8; President *Y*4,

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*Y*11 and *Y*12; Majestic *X*1 and *X*2. The virus inoculations of each strain were made by rubbing the leaves of the healthy plants with the forefinger wetted with infective sap obtained from crushed tobacco plants.

Technique of P. infestans *inoculations*

A strain of the group A of *P. infestans* commonly found on most varieties was used for our experiments. The culture which had been originally isolated from a single zoospore was known to spore freely. Two methods were used to compare the development of the fungus on virus-free and virus-infected plants.

(1) *The whole-leaf test.* This consisted of inoculating detached leaves with a dense zoospore suspension (about 3000 per drop) and then placing them under optimum conditions for development and observation of infection. The method was as follows:

Moist chambers were made by covering the bottom of each of several shallow dishes with moistened cellulose wadding and a perforated sheet of blotting paper. The leaves to be tested were laid on the blotting paper with the petiole pushed through the perforations to make close contact with the moistened cellulose layer. Then, using a pipette, three drops of zoospore suspension were put on each leaflet. Usually the drop did not spread over the leaflet surfaces, and so the inoculation spots could be observed for some days with a binocular microscope. After inoculation the dishes were covered with glass sheets and placed out of direct sunlight. Under these conditions with temperatures varying from 18 to 23° C. the leaves remained fresh for 6–8 days.

Observations were made daily and changes caused by *P. infestans* noted. The most important changes were in the time that elapsed before the first sporangiophores were visible and in the speed of development in the succeeding days. The behaviour of the parasite on the different samples was compared by numerical values assigned to degrees of fructification as indicated in the following:

Degree of fructification	Numerical value
No fructification	0·00
Occasional sporangiophores	0·25
Occasional small patches of sporangiophores	0·50
Several small patches of sporangiophores	1·00
Several large patches of sporangiophores	2·00
Large patches of sporangiophores and leaflet almost destroyed	3·00

The 'index of fructification' is the average of the total single values.

(2) *The leaf-piece test.* This test, to be described by Haigh and Müller in a subsequent paper, was applied as follows:

Small circular pieces were stamped out from the leaflets of the plants to be tested with a sharp cork borer. These pieces, distributed at random, were placed in the dishes prepared as described above. A weak zoospore suspension was then uniformly sprayed over such preparations with an atomizer. Some leaf-pieces escaped

infection in these experiments because of the low zoospore concentration used; consequently in the determination of the 'index of fructification' only the infected pieces were taken into account. Five classes were distinguished in these tests.

Degree of fructification	Numerical value
No fructification	0·00
Some sporangiophores	0·25
Scattered fructification	0·50
Surface almost wholly covered with sporangiophores	1·00
Whole surface covered with profuse fructification	2·00

Although measuring the fructification in this manner is rather artificial, differences between the index of fructification exceeding 0·5 were significant.

All *P. infestans* inoculations, unless stated to the contrary, were on the upper surfaces of the leaves. After the removal of leaflets from the various plants for the *P. infestans* inoculations, leaflets were also removed from similar parts of those same plants for inoculation to tobacco to test for virus.

Experimental results

Sporangiophores developed on all leaves used in the experiments with primary virus symptoms except one, an Arran Victory *Y* 5 inoculation, which was severely damaged by virus leaf necrosis. Each figure appearing in the columns under the heading 'index of fructification' in Table 1 is an average of the number of inoculations for each variety and strain as measured by the foregoing standards. That the presence of virus in potato leaves may markedly retard the development of *P. infestans* by the reduced sporangiophore production is shown in Table 1. The experiments also indicate that this reduced fructification is closely associated with the severity of the virus symptoms.

The tests for virus showed that the Katahdin *Y* 5 plants were virus-free in that region of the plant from which leaflets were removed for the *P. infestans* inoculations, despite symptoms of infection on the lower leaves.

Table 2 shows that blight is also retarded in potato plants raised from virus-infected tubers, and that, as in plants recently infected, the reduction in fructification is correlated with the severity of symptoms caused by the viruses. The Craigs Alliance plants numbered *Y* 7(*a*) were virus-free; the parent plants were killed within a month of infection and the tubers probably remained healthy.

Virus X host reaction

Infection with virus *X* retarded development of *P. infestans* in a similar way to virus *Y*. The decrease of blight fructification as systemic virus symptoms increased in severity is the same whether the virus concerned be *X* or *Y*. We also found that if all the leaves on test were left long enough, the intensity of the fructification became much the same in both virus-free and virus-infected leaves except for those severely affected by virus in which differences remained.

TABLE 1. *Whole leaf tests. Virus Y strains and* Phytophthora infestans. *Primary virus symptoms on plants*

Variety	Virus strain	Virus symptoms	Index of fructification	No. of inoculations
King Edward	*Y*3	No symptoms	After 2 days 0·4; after 3 days 2·2	21
	*Y*5	Severe mosaic	After 2 days 0·0; after 3 days 0·4	25
	*Y*6	Severe mosaic	After 2 days 0·0; after 3 days 1·4	18
	Control	No symptoms	After 2 days 0·6; after 3 days 2·8	16
Arran Victory	*Y*5	Severe mosaic	After 3 days 0·3	15
	*Y*6	Severe mosaic	After 3 days 0·7	15
	Control	No symptoms	After 3 days 2·0	10
Katahdin	*Y*5	No symptoms	After 3 days 2·2	15
	*Y*6	Mild mosaic and veinal necrosis	After 3 days 1·1	15
	Control	No symptoms	After 3 days 2·0	10
President	*Y*5	Veinal and leaf necrosis	After 4 days 0·1; after 6 days 1·4	12
	*Y*6	Severe mosaic and leaf necrosis	After 4 days 0·0; after 6 days 1·4	12
	Control	No symptoms	After 4 days 0·8; after 6 days 2·9	9

TABLE 2. *Whole-leaf tests. With strains of viruses X and Y and* Phytophthora infestans. *Secondary virus symptoms on plants*

			Index of fructification					
			After 4 days		After 5 days		After 6 days	
Variety	Virus strain	Virus symptoms	Upper surface	Lower surface	Upper surface	Lower surface	lower surface	No. of inoculations
British Queen	*Y*7	Severe mosaic	0·0	0·1	0·0	1·6	—	5
	*Y*8	Mild mosaic	0·0	0·5	0·2	2·4	—	5
	*Y*12	Severe mosaic and distortion	0·0	0·0	0·0	0·6	—	8
	Control	No symptoms	0·3	0·6	1·0	2·6	—	5
British Queen	*Y*7	Severe mosaic	—	0·2	—	1·25	—	10
	*Y*12	Severe mosaic and distortion	—	0·1	—	0·2	—	12
	Control	No symptoms	—	2·1	—	3·0	—	10
Craigs Alliance	*Y*1	Slight mottle	0·0	0·1	0·1	2·0	2·7	6
	*Y*7(*a*)	No symptoms	0·0	0·3	0·3	2·7	3·0	3
	*Y*7(*b*)	Severe and intense mosaic	0·0	0·0	0·0	0·0	0·5	6
	*Y*8	Slight mottle	0·0	0·4	0·3	2·3	2·7	7
	Control	No symptoms	0·0	0·3	0·3	2·25	2·8	6
Majestic	*X*1	Necrosis and mild to severe mosaic	—	0·2	—	—	—	15
	*X*2	Slight mottle	—	1·3	—	—	—	17
	Control	No symptoms	—	2·0	—	—	—	16

The leaf-piece tests

Results so far have shown a clear retardation of *P. infestans* development on virus-infected leaves, but the method used was a little crude and did not lend itself to more detailed examination, interpretation or analysis. There are several objections to this 'whole-leaf test' method. The zoospore suspensions were highly concentrated, and it was therefore mainly the speed of fungus spread within the host tissues that was being measured. Moreover, we found that the fungus often behaved rather differently on the various leaves of the same plant and even on different parts of the same leaflet. On the other hand, the use of the 'leaf-piece' test method increased the significance of the 'indices of fructification' provided

TABLE 3. *Leaf-piece tests with virus Y strains and* Phytophthora infestans. *Secondary virus symptoms on President*

Virus strain	Virus symptoms	Days after inoculation: 5 *A*	5 *B*	6 *A*	6 *B*	7 *A*	7 *B*	8 *A*	8 *B*	*N*
Control	No symptoms	78	0·7	83	1·3	84	1·3	84	1·3	87
*Y*4	Slight mottle	71	0·7	77	1·3	77	1·2	79	1·3	87
*Y*11	Mild to severe mosaic	60	0·6	64	1·0	64	1·0	64	1·1	87
*Y*12	Severe mosaic and necrosis	25	0·3	31	0·5	33	0·5	35	0·5	52

A = percentage showing fructification.
B = index of fructification.
N = number of inoculated pieces.

enough pieces were used. Furthermore, by using this method, we did get some indication as to why the development of *P. infestans* is retarded in virus-infected plants. The most important feature of Table 3 is the comparative effect of *P. infestans* on President infected with *Y* 12. Not only was the fructification much reduced on these *Y* 12 leaflets but there was also a considerable reduction in the relative number of leaf-pieces which became infected by the fungus. From this we can suggest that an infection with a virus strain 'severe' to a specific variety may increase the resistance to *P. infestans* infection. The experiments given in Table 4 were carried out to show that the differences between the 'indices of fructification' on healthy and on virus-diseased leaves still remain if we inoculate on the lower surfaces of the pieces.

As already noted we found differences in fructification on leaves of the same plants, and so to determine whether a descending scale of infectivity of plant parts existed we carried out the following 'leaf-piece test' experiment (Table 5 and Fig. 1). Leaf-pieces were cut out of all the leaflets of each of six leaves, including the lowest and highest leaves with the others ranging between, of a virus-free President

TABLE 4. *Indices of fructification in leaf-piece tests. Leaves infected with virus Y strains and inoculated with* Phytophthora infestans *on the lower surface. Secondary virus symptoms on British Queen*

	Days after inoculation			
Virus	4	5	6	*N*
*Y*7	0·66	1·42	1·57	28
*Y*12	0·89	1·55	1·52	27
Control	1·65	1·79	1·89	28

N = number of pieces inoculated.

TABLE 5. *Leaf-piece tests with virus Y and* Phytophthora infestans *comparing the development of fructification on leaves on different parts of the plant. Secondary virus symptoms on President*

			Days after inoculation								
			5		6		7		8		
Virus strain	Virus symptoms	Leaf	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*N*
Control	No symptoms	*a*	33	0·5	50	0·75	50	0·75	50	0·75	12
		b	75	0·6	75	1·1	81	1·1	81	1·1	16
		c	68	0·6	68	1·2	68	1·2	68	1·2	16
		d	95	0·6	100	1·3	100	1·3	100	1·4	20
		e	94	0·8	100	1·4	100	1·4	100	1·4	18
		f	100	1·0	100	2·0	100	2·0	100	2·0	12
*Y*12	Severe	*a*	0	—	0	—	0	—	0	—	11
	mosaic and	*b*	30	0·3	30	0·5	30	0·5	30	0·5	10
	necrosis	*c*	0	—	8	0·25	17	0·25	17	0·25	12
		d	25	0·25	42	0·4	42	0·4	50	0·6	12
		e	100	0·25	100	0·4	100	0·5	100	0·5	5
		f	100	0·4	100	0·75	100	0·75	100	0·75	2

A = percentage of infected pieces. *B* = index of fructification. *N* = number of pieces inoculated.

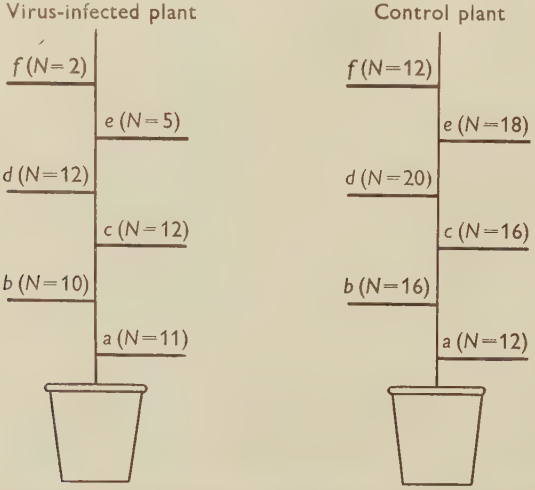

Fig. 1. Position of leaves and number of leaf-pieces (*N*) which were tested as shown in Table 5.

plant and a virus *Y* 12 infected plant of the same variety, and treated in the usual way for these tests. The changes that the constitution of the plant cells undergo with ageing are reflected within a genotype by the ease with which leaves of varying ages in a single plant can become infected by *P. infestans* (Haigh & Müller, to be published in a subsequent paper). As shown in Table 5, there is a marked falling off of infection with increase in age of the leaves of the virus-free plant up to 50 % as indicated by a comparison of the bottom leaf *a* and the top leaf *f*. But the effect of the combination of severe virus infection plus the natural falling off as age increases, makes an even more interesting comparison between the leaves of varying ages on the *Y* 12-infected plant. This combination of influences on the development of *P. infestans* in the field may have a very important bearing upon the epidemiology of this fungus.

Discussion

When the investigations were started to test our observations that virus-infected plants in the field are less affected by blight than healthy plants, we expected to find that the cause was due to constitutional changes in the host. The results of our experiments appear to confirm this. However, this does not necessarily exclude the possibility that the reduced foliage of virus-infected plants induces a micro-climate around the plant which depresses the development of the fungus. This can perhaps be considered as an additional factor which may also decrease the rate of spread of the fungus within the crop. At first sight it may appear difficult to understand how such relatively small differences can show this retardation of 'blight' development in virus-infected plants as compared with virus-free plants. It should, however, be appreciated that a multiplication of four to six generations is needed to create an epidemic from a few scattered sources. On theoretical considerations it can be deduced that a lengthened incubation and a reduced speed of fructification are sufficient to postpone or prevent an epidemic of blight. If, for example, there was a reduction in the fructification rate of 20 % per generation and an increase of incubation time of 25 %, we should find after the sixth generation the difference of blight distribution between healthy and virus diseased plots to be in the ratio of 100:8, provided every zoospore had the same statistical significance. When we compared the clinical changes caused by viruses in potato plants with the development of 'blight' on such plants we found that the more severe the effect of virus the greater was the retardation of 'blight'. This is evident from the results shown in Table 6.

Though the behaviour of *P. infestans* on potato plants may be similarly modified by different viruses and by different strains of the same virus, fructification is closely correlated with the sensitivity of a variety to such strains within a virus. We must therefore conclude that the influence of the virus on the fungus is through the effect of the virus on the host protoplasm which controls the development of the fungus.

To explain the influence of virus infections on blight development from a more physiological point of view is much more difficult, because our knowledge of the nutritional requirements of *P. infestans* is rather slight. There are, however, several features which enable us to consider the factors involved in the retardation of the fungus development. We did not find an increased sensitivity to *P. infestans* to be such a factor. In our experiments we could not find any indication of a negative correlation between the speed of reaction to blight in virus-free and in virus-infected plants. Apparently the factor or factors involved, changeable by virus, have only

TABLE 6. *The relation between sensitivity of host to virus and the delaying effect of virus on fructification of* Phytophthora infestans (*whole-leaf tests only*)

	Virus symptoms					
D	No symptoms	Mild mosaic	Mild mosaic and veinal necrosis	Severe mosaic	Severe mosaic and leaf necrosis	Veinal and leaf necrosis
0	*a d e*	—	—	—	—	—
	g k	—	—	—	—	—
41–60	—	—	*f*	*l*	—	—
61–80	—	*m*	—	*i*	—	—
81–100	—	—	—	*h l*	—	—
				n	*c*	*b*

$$D = \frac{100(B_1 - B_2)}{B_1}.$$

B_1 = 'index of fructification' of the leaf controls.
B_2 = 'index of fructification' of the virus-infected leaves.

a = President, control
b = President, virus *Y*5
c = President, virus *Y*6
d = Katahdin, control
e = Katahdin, virus *Y*5
f = Katahdin, virus *Y*6
g = Arran Victory, control
h = Arran Victory, virus *Y*5
i = Arran Victory, virus *Y*6
k = British Queen, control
l = British Queen, virus *Y*7
m = British Queen, virus *Y*8
n = British Queen, virus *Y*12

a–i, observations after 4 days.
k–n, observations after 5 days.

a passive effect on *P. infestans* in virus-infected plants. This was indicated by the relatively lower numbers of leaf-pieces containing the virus which were infected by the fungus as compared with the healthy virus-free pieces. It may be therefore that the mechanical structure of the epidermal cells is altered by the virus in such a way that penetration of the zoospore germ tubes into the host tissues is rendered much more difficult. This, however, cannot be the only reason, because the speed of fungus development on the virus-infected leaves is also decreased. There must be another factor changeable by virus that influences 'blight' development and this may be nutritional. This suggestion is supported by experimental results with pure cultures which have shown that the growth of *P. infestans* is chiefly controlled by

certain protein constituents which must be available to the fungus for optimal development. Moreover, we know that viruses affect the protein metabolism of the host plant, and so it is conceivable that certain constituents of the host plants, essential to *P. infestans* optimal development, are eliminated or decreased by virus infection. If this is so, we must then conclude that the higher the sensitivity of the host plant to the virus in question the greater are the changes of its protein metabolism with regard to the needs of *P. infestans*.

Our results seem to point clearly to the use of virus-free material whenever possible for the testing of blight resistance.

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SOME PROPERTIES OF BROAD-BEAN MOTTLE VIRUS

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(With Plate 22)

A severe disease affecting many plants in a crop of broad beans was found to be caused by a previously undescribed virus, provisionally named broad-bean mottle virus. The distribution of diseased plants suggested spread by a vector, but none of the six insects tested transmitted it. The virus was transmitted to several species of leguminous plants by mechanical inoculation of sap; infectivity for some hosts seemed to be increased by propagation in these hosts.

The virus has an unusual combination of properties. Its thermal inactivation point is about 95° C., whereas sap becomes non-infective within 3 weeks at room temperature. The infection end-point of broad-bean sap is 1/1000, only a little higher than the precipitation titre with specific antiserum. Precipitation with antiserum occurs over a smaller range of antigen/antibody ratios than with other viruses previously studied, possibly because of its greater solubility; it is not precipitated with $(NH_4)_2SO_4$ until the salt concentration exceeds 75 % saturation.

A specific nucleoprotein, containing nucleic acid of the ribose type, can be isolated from infective broad-bean sap in yields up to 2 g./l. Purified preparations, made by salt precipitation and ultracentrifugation, contain uniform spherical particles approximately 17 mμ in diameter. It is suggested that much of this nucleoprotein is non-infective, but may otherwise resemble infective particles.

In July 1947 we were asked to examine a field crop of broad beans (*Vicia faba*) in Nottinghamshire which was rendered almost worthless by what seemed to be a previously unrecorded disease. More than three-quarters of the plants showed leaf symptoms, consisting of mottling, necrosis and crinkling, but individual plants were affected to different extents. Some were much dwarfed, had produced few or no flowers, and all their leaves were affected, whereas others were more vigorous and showed symptoms on the young leaves only. Diseased plants were often in obvious patches, in which the smallest plants were at the centre and the crippling was progressively less severe towards the periphery. The leaf symptoms suggested that the cause was a virus, and the distribution of diseased plants suggested that it had spread within the crop from some initially infected plants distributed more or less at random.

The disease was readily shown to be infectious by inoculating sap expressed from affected plants to broad-bean seedlings raised under glass, when the seedlings soon developed leaf symptoms similar to those shown by the field plants. Seedlings inoculated with sap from different field plants all developed similar symptoms, and we have no evidence that more than one virus was involved in the outbreak. This

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virus seems to differ from any previously described, and we propose to call it broad-bean mottle virus (BBMV). Our studies have failed to explain the outbreak, for we have no information on the source of the virus or the method whereby it spread, but they have shown it to have some unusual properties that justify recording.

Symptomatology and host range

No attempt was made to determine the complete host range of BBMV, but transmissions were attempted to several different species. The inoculum was freshly expressed sap from diseased broad-bean leaves; this was rubbed over leaves of the test plants which were first lightly dusted with either a diatomaceous earth or 400-mesh carborundum. No infection occurred in *Datura stramonium* L., *Lycopersicum esculentum* Mill., *Medicago sativa* L., *Nicotiana tabacum* L., *N. glutinosa* L. or *Vicia sativa* L. In addition to *Vicia faba* L., other species found to be susceptible were *Pisum sativum* L. (edible pea), *Lathyrus odoratus* L. (sweet pea), *Phaseolus vulgaris* L. (French bean), *Trifolium incarnatum* L. (crimson clover), *T. pratense* L. (red clover) (Pl. 22, fig. 1), *T. subterraneum* (subterranean clover) and *Soja max* Piper (soy bean).

Two varieties of broad bean, Prolific Big-pod and Broad Windsor, were used and reacted similarly. The inoculated leaves developed no obvious lesions, and the first visible symptom was a clearing of the veins of the youngest leaves, which appeared 8–10 days after inoculation (Pl. 22, fig. 2). Subsequent symptoms varied with the season. At all seasons, the vein-clearing faded to be replaced by a bright interveinal mottle, but during winter there was also extensive necrosis; the leaves blackened first at the margins and then over the whole laminae, and the main growing point and axillary shoots were also killed. When the virus was first obtained from the field it caused these necrotic symptoms on glasshouse plants during the summer, but with continued transfer to plants under glass, the culture seems to have become less virulent; after a year in glasshouse-grown plants, it caused necrosis during the winter only, and now it causes only mosaic symptoms in broad bean throughout the year. The leaves develop a bright yellow interveinal mottle, with some distortion, and the whole plant is considerably reduced in size (Pl. 22, fig. 4).

The virus caused a lethal disease in the edible pea. The inoculated leaves became necrotic and shrivelled in 6–8 days, and systemic infection led to a wilt and collapse of the whole plant, the young leaves of which sometimes showed a faint mottle before wilting. Sweet-pea plants were not killed, but infected plants were severely stunted and the leaves were mottled and reduced in size.

In attempts to discover a host in which BBMV produced local lesions suitable for quantitative infectivity tests, many varieties of French bean were inoculated. All proved susceptible but none gave countable necrotic local lesions. Their reactions fell into one or other of two distinct types, characterized by the varieties

Canadian Wonder and Prince. The first symptom to become obvious in Canadian Wonder was a bright yellowing along the veins of the youngest leaves; this appeared about 12 days after the lower leaves were inoculated and was succeeded by a bright interveinal mottling (Pl. 22, fig. 3). In the variety Prince, systemic symptoms were much less obvious. Some infected plants showed a transient mosaic in the younger leaves, but many behaved as symptomless carriers. The difference between the two types of French bean varieties seemed to be correlated with the concentration of infective virus present in them. Sap from systemically infected leaves of Canadian Wonder plants when diluted 1/10 with water was infective to broad bean without incorporating carborundum powder in the inoculum, whereas sap from similar leaves of Prince plants was infective only when undiluted and inoculated together with carborundum. French bean varieties of both types often produced unusual reactions on the inoculated leaves; a month or so after inoculation, and when systemic symptoms had already been obvious in such varieties as Canadian Wonder for a fortnight, chlorotic spots developed on the inoculated leaves. They were about 2 mm. in diameter, remained discrete and persisted until the death of the leaves (Pl. 22, fig. 5). They were presumably local lesions, and could be counted without difficulty, but they were unsuitable for quantitative work because they developed so slowly that their relation with initial infection sites was uncertain; also they were not always produced.

Crimson clover, red clover and subterranean clover all reacted similarly, becoming systemically infected and showing mosaic symptoms. We have not searched for the virus in clover plants in the field, but as BBMV causes only a mild disease in them, they could provide perennial sources of infection. No systemic infection occurred in soy bean, in which the only lesions were pin-point necrotic spots on the inoculated leaves, too small and formed too erratically to be used for assaying infectivity.

Transmission

The only method we have found of transmitting BBMV is by mechanical inoculation of sap from infected plants. Five species of aphids: *Myzus persicae* (Sulz.), *Acyzthosiphon pisum* (Harris), *Macrosiphum euphorbiae* (Thomas), *Aphis fabae* (Scop.) and *Hyperomyzus tulipaellus* (Theobald) were tested as possible vectors, but none transmitted the virus. In some tests the aphids were left to feed undisturbed on infected broad-bean leaves for periods up to 2 days before they were transferrred to healthy seedlings, on which they were again left to feed for 2–4 days. In other tests, the aphids were first starved for 4 hr., then fed for periods of 2–5 min. on infected leaves before they were placed on healthy seedlings. If any of these aphid species is a vector, it would seem that BBMV must be a persistent virus (Watson & Roberts 1939) with an incubation period in the insect of many days, a possibility we have not excluded but consider unlikely.

Attempts were also made to transmit with the bean weevil, *Sitona lineatus* (Linn.). One hundred insects caught in the field were caged for 3 days on an infected broad-

bean plant and then transferred in batches of ten to test seedlings, on which they were left for 5 days. All the test plants remained healthy.

The occurrence in the original diseased crop of scattered plants that were much crippled, and had obviously been infected early in their life, suggested that BBMV, like some other viruses in leguminous hosts, might be seed-transmitted. We could get no evidence to support this suggestion. A total of 130 seeds of broad bean, var. Broad Windsor, and thirty of French bean, var. Canadian Wonder, were collected from infected plants and when sown all produced healthy-looking, virus-free seedlings.

Serological and physico-chemical properties

Preliminary tests of the behaviour of BBMV *in vitro* suggested that it possessed an unusual combination of properties. Tests of the effect of dilution on infectivity suggested that the virus content of infective sap was not high, whereas serological tests showed that infective sap contained large quantities of a specific antigen. With other viruses that are readily sap-inoculable, the ratio between the dilution end-points for infectivity and precipitation has been reasonably constant, and with all the end-point for infectivity has greatly exceeded, usually by a factor of more than a hundred, the end-point for precipitation. With BBMV, in striking contrast, the precipitation end-point often approached or equalled the dilution end-point for infectivity. Sap from infected broad bean, for example, sometimes precipitated up to dilutions of 1/640 but failed to cause infection, unless celite or carborundum powder was incorporated in the inoculum, at 1/1000. Similarly, sap from systemically infected Canadian Wonder French bean precipitated up to dilutions of 1 64 but failed to infect at dilutions over 1/100.

Another unusual feature was that the dilution end-point for infectivity depended both on the species of infected plant and species of test plant. Sap from French bean, infective for French bean at dilutions up to 1/100, would not infect broad bean when diluted more than 1/10, and sap from broad bean, infective for broad bean at dilutions up to 1/1000, would not infect French bean when diluted more than 1/100. The only explanation of this phenomenon we can offer is that the virus occurs in forms that differ in their ability to infect the two hosts, and that propagation in either host encourages a greater proportion of the form infective for that one. Prolonged propagation in a series of plants of either host, however, did not destroy ability to infect the other, or apparently reduce this ability to a level lower than that found in the first plant of the series. It seems unlikely that the phenomenon is explicable on the basis of each host containing inhibitors of infectivity that act specifically on the other, for the effects of inhibitors are reduced by dilution, and mixtures of viruses and inhibitors non-infective when concentrated usually become infective when sufficiently diluted.

Antisera that reacted with an antigen specific to the sap of infected plants were prepared by injecting rabbits with infective sap clarified by heating to 60° C. and then centrifuging for 10 min. at 6000 r.p.m. Four antisera were prepared in this

manner, one against sap from Canadian Wonder French bean and three against sap from broad bean. Six injections of 2 ml. of clarified sap were given, and the rabbits were bled 10 days after the last injection. In addition to reacting with infective sap, all four antisera contained antibodies that precipitated components from the sap of healthy plants, of both French bean and broad bean, showing that the two species contain common antigens. The precipitin titres of the antisera varied from 1/80 to 1/320, considerably less than the titres to be expected from injecting comparable amounts of tobacco mosaic or tomato bushy stunt virus. Although the antigen specific to the plants infected with BBMV seems less active in stimulating antibody production than these other viruses, it is more active than the antigens present in sap from uninfected bean plants. When tested against sap from uninfected plants, none of the antisera precipitated when diluted further than 1/20. Thus, without the necessity of adsorbing them with sap from uninfected plants, these sera could be used specifically for infective sap merely by using them at dilutions greater than 1/30. A further antiserum that did not precipitate any components from sap of healthy plants was produced by injecting a rabbit with a purified virus preparation made by the method described below. With other viruses there is much evidence relating the antigens specific to infected plants with the viruses themselves. There is less evidence for this relationship with BBMV, but for the sake of simplicity we shall refer to the antigen as the virus, although much of it may well be non-infective.

Precipitin tests were made by mixing 1 ml. of antigen solutions with 1 ml. of diluted antiserum in tubes of 7 mm. diameter, which were placed in a water-bath held at 50° C. In these conditions when infective broad-bean sap is titrated against antiserum at a dilution of 1/40, precipitation starts in about 10 min. with a clearly marked optimum, floccules appearing first in the tubes containing sap at dilutions around 1/80. The floccules are dense and soon settle into compact aggregates at the bottom of the tube, the reaction being characteristic of a somatic-type antigen and suggesting that the particles are probably isometric. Precipitation is complete in about 2 hr. when it usually occurs over a range of dilutions from 1/40 to 1/320 or 1/640. This is a narrower zone of precipitation, with much more pronounced inhibition by antigen excess, than occurs with other viruses we have tested, even with the somatic type such as tomato bushy stunt, and more closely resembles the precipitin behaviour of serum proteins. As will be described below, BBMV is more soluble than other plant viruses whose properties have been studied, and this fact probably accounts for its different behaviour in precipitin tests; if BBMV contains more lyophilic groups than the other viruses, then it will need to combine with more antibody before it becomes insoluble.

Tests of the stability of BBMV *in vitro* also indicated unusual behaviour. The thermal inactivation point was determined by heating 2 ml. samples of infective sap from broad bean for 10 min. at various temperatures and found to be higher than that previously reported for any virus. As shown in Table 1 temperatures

higher than 95° C. are needed to cause complete inactivation of infectivity and serological activity. The two activities have the same inactivation point, but the precipitin tests, made on heated fluids clarified by centrifugation, suggest that an increasing proportion of antigen is destroyed at temperatures between 85 and 95° C. With no method of making accurate assays for infectivity, there is no unequivocal evidence that the two activities are affected to the same extent at these lower temperatures, but it seems probable that loss of infectivity and serological activity are correlated. The reduction in precipitation end-point of sap heated between 85 and 95° C. is not caused by loss of antigen separating with the coagulum of denatured normal plant protein, for essentially similar results are obtained by heating purified virus preparations. When these are heated at about pH 6 in the presence of a little salt, precipitates separate at temperatures above 80° C.; their size increases with temperature up to 95° C. and is roughly proportional to the fall in precipitin titre of the supernatant fluids. When salt-free preparations are heated between 80 and 95° C., they become slightly opalescent, but no precipitate of denatured protein separates until a little salt is added.

TABLE 1. *Effect of heat on broad-bean mottle virus*

Temperature (° C.)	60	70	80	85	90	95	100
Infectivity (no. of plants infected)/(no. inoculated)	10/10	10/10	10/10	16/16	14/16	3/16	0/16
Precipitation end-point titre	1/320	1/320	1/160	1/80	1/20	1/5	No precipitation

2 ml. of infective sap were heated for 10 min. at the specified temperature.

In sharp contrast to its high thermal inactivation point, BBMV soon loses infectivity on ageing *in vitro*. Sap from broad bean, infective at a dilution of 1/1000 when freshly expressed, was non-infective after 20 days at 15° C. Unlike the loss of infectivity on heating, that occasioned by ageing is not accompanied by a corresponding loss of serological activity; sap from infected plants, and purified virus preparations, retain their precipitin titres unchanged over periods of many months.

Attempts to concentrate the virus by precipitation from clarified sap with ammonium sulphate demonstrated its greater solubility in salt solutions than other plant viruses previously studied. All these have been precipitated by concentrations of ammonium sulphate less than one-third saturated. Additions of various amounts of the salt to sap from infected broad bean, followed by low-speed centrifugation, showed that BBMV, whether assayed by serological or infectivity tests, did not begin to precipitate until the salt concentration reached 70 % saturation, and precipitation was not complete under 80 %. Much normal plant protein is precipitated at 40–50 % saturation and the greater solubility in ammonium sulphate of BBMV provides a simple method of fractionation.

The purification of the virus, however, is complicated by the production in the

bean sap of black pigment that adheres tenaciously to all the protein fractions that can be separated by precipitation with ammonium sulphate. Sap freshly expressed from broad-bean leaves and clarified by centrifugation for 10 min. at 8000 r.p.m. is clear and light brown, but if left to stand it soon begins to become dark, first at the surface and then through the whole fluid which in a few hours resembles ink. This is presumably caused by the oxidation of tyrosine or 3:4-dihydroxy-phenylalanine to melanin (Raper, 1932); it can be delayed for a time by 0·5 % Na_2SO_3, but this treatment inactivates the virus. Protein fractions precipitated from freshly expressed sap by ammonium sulphate are at first white, but when redissolved in water they soon blacken, as also do precipitates produced by the addition of alcohol or material sedimented by ultracentrifugation. The only method we have found for removing the black pigment is by treatment with activated charcoal, which is fully effective if applied at the correct time during the course of preparation.

We have usually found the following method to give colourless preparations of the virus. Infective sap from broad-bean leaves with mosaic symptoms is clarified by centrifugation at 8000 r.p.m., and the supernatant fluid mixed with an equal volume of saturated ammonium sulphate solution, when a dense white precipitate separates. This is removed by centrifugation, and solid ammonium sulphate is added to the supernatant fluid to bring the salt concentration to 80 %, when another bulky white precipitate separates. (Sap from uninfected beans also gives a large precipitate at half saturation, but after this treatment gives only a small precipitate with 80 % saturation.) The mixture is centrifuged after standing for a few minutes, and the supernatant fluid discarded. The precipitate dissolves immediately when stirred with its own volume of water, and now starts to blacken. The solution is diluted to the original volume of sap and then activated charcoal is added at the rate of 3 g./100 ml. After thorough mixing, the charcoal is removed by centrifugation, leaving a clear solution which is usually colourless by transmitted light but has a bluish tinge by reflected light. If it is still darkly coloured, or if it darkens on standing, it is again treated with charcoal. Applying the charcoal direct to infective sap or at later stages when the preparations have been black for some time, is less effective and rarely produces colourless preparations.

After removing the charcoal, the supernatant fluid is again brought to 80 % saturation with ammonium sulphate, and the precipitate centrifuged off. It is taken up in one-quarter the volume of the original sap and centrifuged to free it from any insoluble material. If there is an appreciable quantity of this, the fluid is diluted 1/4 with water and the precipitation with ammonium sulphate repeated until all the material that precipitates dissolves rapidly in water. The solution is then dialysed to free it from salts; if the treatment with charcoal has not given a colourless preparation, dialysis does not remove the pigment.

Serological assays at different stages during the course of purification show that the method of purification leads to little loss of antigen and recovers about 80 % of that initially present in infective sap. The yields of purified material indicate

that infective sap from broad bean usually contains between 1·5 and 2 mg. of virus per ml., almost as much as the amount of tobacco mosaic virus in infective tobacco sap. When titrated against antiserum, specific precipitation occurs down to concentrations of 10 mg./l., about four times the amount needed with tomato bushy stunt or tobacco necrosis viruses, whose serological behaviour BBMV most closely approaches. This difference does not entail the assumption that only a quarter of the material in the preparations is the specific antigen; it is more likely a reflexion of the much greater solubility of BBMV. Freshly made preparations are infective, but seem to be less so than sap containing the same weight of antigen, although accurate comparisons have not been made. To infect broad bean, inoculum needs to contain from 0·1 to 0·01 mg./ml.

We have not made extensive tests for heterogeneity in the purified preparations, but they do not seem to contain a mixture of particles with widely different characters. When examined in the electron microscope, the only material detectable has uniformly spherical particles with a diameter about 17 mμ (Pl. 22, fig. 6). When ultracentrifuged, no further fractionation is obtained; the sedimented pellet is a clear jelly, which dissolves immediately when stirred with water. If ultracentrifuged in conditions in which only a part of the preparation sediments into a pellet, the sedimented and non-sedimented parts behave similarly in precipitin tests, look alike in the electron microscope, and have similar phosphorus and carbohydrate contents.

The purified preparations are largely, and probably exclusively, nucleoprotein; their nitrogen, phosphorus and carbohydrate contents are about 15·5, 1·6 and 6·5 % respectively, similar to values for tomato bushy stunt and tobacco necrosis viruses (Bawden & Pirie, 1938, 1942). About 80 % of the phosphorus originally present can be recovered in the form of a protein-free nucleic acid by treatment with cold alkali in the manner described by Johnson & Harkins (1929) for the isolation of yeast nucleic acid. This gives a positive Bial's test for pentoses and is hydrolysed by pancreatic ribonuclease, showing that, like all other plant viruses so far purified, BBMV contains a nucleic acid of the ribose type.

Like tomato bushy stunt and the tobacco necrosis viruses, BBMV is soluble over the whole pH range in which it is stable. In the presence of a little salt it precipitates irreversibly at pH values around 2. Unlike these viruses, its solubility in ammonium sulphate solutions between 0 and 20° C. is not increased by cooling; preparations in 50 and 60 % saturated ammonium sulphate solution were water clear at 20°, but the first became opalescent when cooled to 2° and the second developed a precipitate.

Discussion

Our results show that plants infected with BBMV contain large amounts of a specific antigen, which can be isolated as a stable nucleoprotein. The main problem that calls for discussion is the relationship between this nucleoprotein and

the virus itself. With other plant viruses that have been studied, there is good evidence identifying them closely with the antigens specific to infected plants, and by analogy we have referred to this antigen as BBMV. In view of the unusually low ratio of infectivity to serological activity, however, the analogy may well be unjustified. When tested on a common host, French bean, the initiation of infection calls for inocula containing more than a thousand times as much antigen with BBMV as with the Rothamsted tobacco necrosis virus. Unfortunately, the significance of this fact is not obvious; it is certainly unsafe to assume that preparations of BBMV therefore contain less than one-thousandth the number of potentially infective particles, for infectivity is not an absolute character of virus preparations but depends on the host plant. Preparations of tobacco mosaic virus, for example, need to be much more concentrated to infect French bean than to infect tobacco or *Nicotiana glutinosa*.

No definite interpretation for the low infectivity of BBMV per unit weight of antigen can be advanced, but it seems likely that much of it is non-infective. In this respect BBMV does not necessarily differ qualitatively from other viruses that have been studied, but only quantitatively. To gain infection with other viruses that have been purified, even the most sensitive hosts need to be inoculated with many thousands of particles. This can be accounted for in part by wastage, but there is good evidence that preparations of several viruses, despite their seeming homogeneity by many tests, do consist of mixtures of infective and non-infective material. Bawden & Pirie (1945*a*) have fractionated the material with the serological specificity of tobacco mosaic virus into fractions with widely different infectivities. They (1945*a*, 1950*a*) have also shown that sap from plants infected with the Rothamsted tobacco necrosis virus contains a mixture of specific nucleoproteins, with obscure interrelations, and whose infectivity is greatly affected by the environment. Likewise, Markham & Smith (1949) have demonstrated two specific proteins, indistinguishable serologically or by the electron microscope, in sap from plants infected with turnip yellow mosaic virus, only one of which seems to be a nucleoprotein and able to initiate infection.

Preparations of BBMV probably provide another example of the occurrence of mixtures of related substances only some of which are infective. It seems likely that, in size and gross constitution, infective particles resemble the others, and that small differences determine ability to initiate infection. With BBMV, as with the others, there is no definite evidence about the origins of the various types of anomalous proteins. With other viruses, three possibilities have been suggested (Bawden & Pirie, 1950*b*). One is that non-infective material is immature virus, particles not completely synthesized to the state of becoming infective; a second is that infection leads to the synthesis of a range of related but not identical particles, only some of which are infective; the third is that the non-infective material is virus particles changed in some manner that has destroyed infectivity. The third possibility is obviously very likely to apply to BBMV, which rapidly loses infectivity on ageing

in vitro without seeming to undergo any other gross change in properties. If inactivation proceeds *in vivo* as it does *in vitro*, then sap would inevitably contain mixtures of infective and non-infective particles. The greater susceptibility of BBMV than other viruses to this type of inactivation could account for the greater ratio of specific antigen to infectivity, but the other possibilities are not excluded because the third is the more plausible.

In the diseased crop from which BBMV was first obtained, there was good visual evidence to suspect that it has a vector. As the virus is apparently not seed-transmitted in broad bean, it must have been introduced into the crop from some outside source, and the distribution of affected plants suggested spread within the crop. The virus is not so easily transmitted by inoculation of sap, and the plants were not sufficiently in contact with one another to suspect that spread occurred without a vector. Of other insect-transmitted viruses that have been purified, only squash mosaic (Takahashi & Rawlins, 1947) and turnip yellow mosaic (Markham & Smith, 1949) have spherical particles and some other properties in common with BBMV. Both these viruses have been found to be transmitted by beetles. There is as yet insufficient evidence to suggest that the type of insect vector is correlated with any physico-chemical characteristics of viruses, but, despite our failure to transmit BBMV with *Sitonea lineatus*, its vector may be found in some such insect that feeds by biting leaves rather than by sucking.

We are indebted to Dr A. Kleczkowski for isolating the nucleic acid, to Mr H. L. Nixon for taking the electron micrographs and to Mr N. W. Pirie for doing the ultracentrifugations.

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EXPLANATION OF PLATE 22

Fig. 1. Two red clover (*Trifolium pratense*) leaves infected with broad-bean mottle virus, showing mosaic symptoms.

Fig. 2. A broad-bean leaf showing vein clearing. Photographed 10 days after the plant was inoculated with broad-bean mottle virus.

Fig. 3. A leaf of French bean, var. Canadian Wonder Improved, with bright yellow mottle. Photographed 6 weeks after infection with broad-bean mottle virus.

Fig. 4. A broad bean leaf infected with broad-bean mottle showing intense yellow mottle.

Fig. 5. A primary leaf of French bean, var. Bountiful, showing discrete chlorotic local lesions. These developed a month after inoculation with broad-bean mottle virus.

Fig. 6. Electron micrograph showing spherical particles 17 mμ in diameter from a purified preparation of broad-bean mottle virus. $\times$40,000.

Photographs 1–5 by Mr V. Stansfield.

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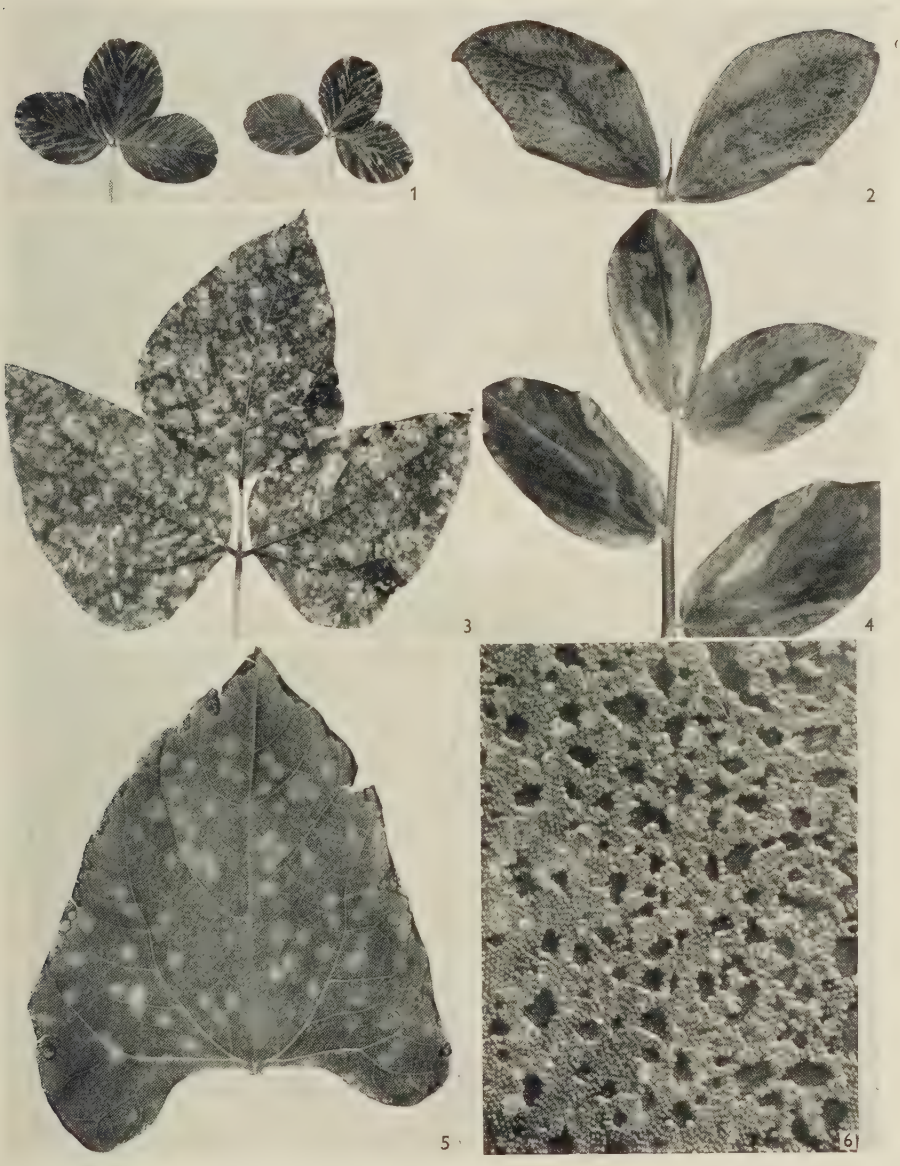

Figs. 1-6.

VIRUS DISEASES OF CACAO IN WEST AFRICA

VIII. THE SEARCH FOR VIRUS-RESISTANT CACAO

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The methods used and the results of 10 years' search for cacao resistant to swollen-shoot disease are described. Selection among the trees surviving in farms devastated by this virus disease led to the discovery of mild virus strains which can protect trees against virulent strains.

When tested by graft inoculation with virulent virus, none of the selections showed any immunity or resistance save that conferred by previous mild-strain infection. A low degree of tolerance was found in some selections.

Local selections and a range of new introductions were tested by mealybug infection, and only types from the Upper Amazon region of Ecuador were consistently resistant to infection. This genetical resistance seems to be strongest in cacao from the Nanay peninsula, near Iquitos.

The search for a virus-immune or resistant type of cacao and the allied studies of host susceptibility and virus variation have always been given an important place in cacao virus research at Tafo. This paper describes two periods in the investigation. In the earlier period, trees found surviving in farms devastated by swollen-shoot disease were selected, propagated by budding and the budlings tested for virus resistance or tolerance by graft-inoculation from severely diseased plants. The selections were, by nature of their origin, mostly of the highly uniform West African Amelonado type. In the later period, individual testing of plants selected in the field was discontinued in favour of extensive testing of the available cacao types for comparative resistance. By this time the vectors and virus-vector relationships had been determined, and insects were used instead of grafts for the transmission of virus.

Resistance is used here in the sense of resistance to primary infection; *tolerance* infers infection with a virulent strain but without associated severe symptoms; a plant which apparently resists infection because of prior infection with a mild strain is said to be *protected* against the strain used in the attempted *superinfection*. Attempts to transmit cacao viruses by inoculation of extracted sap failed. The term *inoculation* will be applied in this paper to the process of infecting plants by patch grafts or by means of insect vectors.

The virus used in the experiments was the economically important cacao virus 1A (Posnette, 1947), of which there are many inter-protecting strains, grading in virulence from severe to mild. The virulent strains cause rapid defoliation of

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susceptible trees and death usually occurs within 2 years of first symptom-expression; the mildest strains, on the other hand, are virtually symptomless in cacao, and have had little effect on host vigour during our investigations.

First period: selection and testing by graft inoculation

(1) *Selection of material*

Swollen shoot had already caused widespread devastation over some 200 square miles before it was shown to be a virus disease (Posnette, 1941). The selection of surviving trees could therefore be begun immediately, and by the end of 1940 318 trees had been tagged and their condition recorded. At first, the criterion for selection was that a tree should be the sole apparently healthy survivor in any area of at least 2 or 3 acres devastated by swollen shoot. This was soon modified to admit the selection of small groups of surviving trees as, under the native system of planting beans straight from the pod, adjacent trees are likely to be sibs, with similar responses to infection. Later, trees with very mild symptoms were selected, as it was thought that they might be tolerant. By the end of 1945, most of the devastated area had been traversed along bush-paths and 452 trees had been selected.

We had intended to record the condition of each selected tree half-yearly and to propagate only the promising ones, but, owing to the rapid regeneration of 'bush' on some abandoned farms and the removal of cacao trees to make way for food crops on others, this system was abandoned and an attempt was made to propagate each selection.

All but ten of the trees selected in devastated farms were West African Amelonado, the type most commonly grown in the Gold Coast. Its uniformity is a serious disadvantage in selection work, so to introduce a wider range, twenty-five of the most vigorous Trinitario trees growing in the Botanic Garden, Aburi, were propagated for testing. A further thirty-five Trinitario clones originally selected for yield (Posnette, 1943) were also tested.

Another attempt to widen the genetical range of cacao under trial was made in 1942, when about 2000 mature trees of Trinitario type were infected by bark-grafting at the abandoned Agricultural Stations of Peki (Eastern Province) and Wiawso (Western Province). The budwood used for grafting was taken from nearby diseased trees. When it was later shown that the viruses used at Peki and Wiawso, besides being too mild for resistance trials, were distinct from virus 1A to which resistance is most desirable, selection work on these plants was curtailed.

(2) *The method and results of testing*

Testing was done at Akwadum in the centre of the heavily infected area of the Eastern Province. Budwood from the selected trees was propagated at first on Amelonado seedling stocks already established in 1941. Seeds were collected from

the selected trees, sown in nursery beds, and the seedlings planted out as rootstocks in 1942 so that scions could be grown on possibly resistant stocks. Up to ten stocks were budded from each selection for the first test. It was intended that both a stock shoot and the scion should be grown on each plant. This was not always done, but wherever stock and scion shoots were established together their reactions to infection were invariably similar.

About half the selections were eliminated because the buds failed to grow; in many of these instances the stock nevertheless became infected with a virulent strain. The other selections were divided into four groups according to scion reaction:

(*a*) weak growth with severe symptoms;

(*b*) good growth with mild symptoms on early flushes; later growth symptomless or with occasional mild symptoms;

(*c*) good growth without symptoms;

(*d*) some scions reacting as under (*a*), others as under (*b*) or (*c*).

The selections of group (*a*) were discarded. Those of groups (*b*) and (*c*) were tested by grafting half the budlings in each clone from source plants with severe symptoms of virus 1 A. This had no visible effect on the clones of group (*b*). Most of the budlings of the symptomless clones (group (*c*)) succumbed in the manner of susceptible plants, but a few developed only mild symptoms.

The clones which reacted favourably in this test were propagated from the non-inoculated plants for a further test in which different virus sources were used. The results of the second test were variable; with some clones, those of group (*b*) and some of group (*c*), the resistance or tolerance shown in the first test was repeated, while others behaved like susceptibles.

These results were interpreted as follows.

No immunity to severe strains of virus 1 A had been discovered save for that associated with prior infection.

Most of the trees selected from farms, though symptomless or with mild symptoms at the time of selection, were infected with a virulent strain. This meant that the trees either were selected during the incubation period of the disease, or possessed a temporary field tolerance.

The selections of group (*b*), which comprised about a tenth of those made from farms, were either permanently and inherently tolerant of virulent strains, or were infected when selected with a mild strain which protected against virulent 1 A. Since plants of this group and some of group (*c*) appear to resist superinfection with virulent strains, the term 'quasi-resistance' will be used to refer to the phenomenon.

The selections of group (*c*) were mixed. Most were susceptible to virulent-strain infection, which indicated that the trees had escaped infection in the field, and so were propagated as healthy susceptible plants. The budlings of the remainder developed mild symptoms in the first test. With some selections, such a response suggested prior mild-strain infection, but this interpretation was inappropriate where, as with many Trinitario selections, the freedom from virus of the parent

trees was indisputable. Clones of the latter category were possibly highly tolerant of virulent-strain infection, or perhaps had even induced a change in virus virulence. The remaining possibility was that the budlings had been fortuitously infected with a mild strain of a virus complex previously dominated by virulent strains. Where a mild response in the first test was succeeded by a severe one in the second, the last explanation seemed the most probable.

The budlings of group (*d*) either differed in their tolerance of virulent-strain infection or had been infected with strains of different virulence.

Further study of the nature of the quasi-resistance shown in groups (*b*) and (*c*) and of variation in virus 1 A were obviously necessary before the results of these resistance tests could be interpreted correctly.

(3) *The nature of quasi-resistance and the complexity of virus 1 A*

An experiment carried out in 1944 showed that the quasi-resistance of a promising clone, 167, was due to protection conferred by mild-strain infection. One-year-old Amelonado seedlings were used as indicator plants. Twelve (group 1) were infected from an untested 167 budling; six (group 2) were infected from a budling of 167 which had been graft-inoculated with a virulent strain 2 years before, but had not developed severe symptoms; six (group 3) were inoculated with a virulent strain, and a further six (group 4) were untreated. All the seedlings in groups 1 and 2 developed mild symptoms and later became symptomless. After 8 months, six of group 1 were inoculated with a virulent strain. The plants of group 3 developed symptoms typical of the virulent strain and died within 2 years, but all the plants in groups 1, 2 and 3 remained free from severe symptoms.

The quasi-resistance of ten budlings representing another seven clones was also investigated. Five of the budlings belonged to three naturally infected clones fully resistant to superinfection with virulent strains of virus 1 A. A further four budlings belonging to three clones developed from virus-free trees, had shown considerable tolerance of virus graft-inoculated from a severely diseased plant. The tenth budling resembled the previous four in origin and response to infection, but the other members of the clone to which it belonged had proved to be highly susceptible. Another budling representing a uniformly susceptible clone was included as a control.

The indicator plants were 1-year-old Amelonado seedlings graft-inoculated in series of eight to twelve, one series to each budling. The indicator plants were observed for a year after inoculation and their reaction to infection designated as mild, intermediate or severe. Mildly affected plants developed sparse symptoms on only occasional growth flushes; plants of intermediate reaction bore mild symptoms on almost every flush and growth was retarded; severely affected plants were stunted, the leaves bore marked symptoms and partial defoliation was usual. The reactions, especially when consistent in a series of plants, were considered to be directly indicative of virus virulence. The results are summarized in Table 1.

Virulent strains were transmitted only from budlings 10 and 11. Budling 10 had survived infection for 6 years and was moderately vigorous at the time of testing, though it has since died; apparently the nature of its quasi-resistance was tolerance of the virulent strain. The result obtained with budling 11 (control) was as expected. The virus infecting budlings 1–9 was clearly mild; in budlings 6–9 it had seemingly evolved or emerged from the same virus or complex, AR 15, which produced severe symptoms in budling 11.

Three months after inoculation, the indicator plants infected from budlings 1–9 were classified thus: (1) no symptoms developed, (2) the first mild symptoms present on the current flush, (3) one or more mild symptom-bearing flushes developed previous to the current symptomless flush. Eight indicator plants of

TABLE 1. *Virulence of strains obtained from budlings showing two kinds of response to infection*

Source of virus	Severely diseased plant used for infection of source	Response of budlings	Response of indicator plants
Budlings 1–5	Natural infection	Mild	Mild
Budlings 6–9	AR 15	Mild	Mild
Budling 10	SS 163	Mild	9/10 severe, 1/10 intermediate
Budling 11	AR 15	Severe	11/12 severe, 1/12 intermediate

group (1), six of group (2) and four of group (3) were reinoculated by grafting from a source of virulent virus with which six virus-free Amelonado seedlings were also inoculated as controls. Within 7 months severe symptoms had developed only on the control plants and on one of the group (1) plants. (This one plant may not have become infected with the mild strain, or, alternatively, the protection may have broken down. Such failure was very rare but it could be induced experimentally by coppicing, and consequently the term 'immunity' has been avoided.) The experiment was continued for over a year without further significant results.

It was clear, then, that the clone 167 and the six clones represented by the budlings 1–5 and 6–9 were resistant to virulent-strain infection because of prior infection with a mild strain (or strains) presumably related to the severe strain. It appeared, too, that a mild strain of virus 1 A would induce this type of resistance in any susceptible cacao plant.

The problem that remained was whether the mild strain had emerged because of a selecting influence inherent in the budlings 6–9 or had merely appeared fortuitously from a complex in which a severe strain had been dominant. This was investigated both by further indexing of virus from apparently tolerant budlings and by a direct test for change of virus influence in tolerant clones.

(1) *Further indexing*. A survey of the budlings which had been raised virus-free and tested for resistance using budwood from the severely diseased source plant AR 15 (i.e. those similar to the budlings 6–9 of the last experiment) revealed a variety of responses. The reaction to infection was not always similar among

budlings of the same clone. Eighteen of these budlings and two infected with strain AR4 were chosen as covering the range of responses, and the virulence of the infecting virus was indexed on year-old Amelonado seedlings. Data concerning the budlings and the results of indexing are given in Table 2.

The virulence of transmitted virus varied from budling to budling. Mild strains were usually obtained predominantly from mildly affected budlings and virulent from severely affected budlings, but virulent and mild strains were also obtained from mildly and severely affected plants respectively. The various plants of a clone

Table 2. *Virus variation in budlings graft-inoculated with apparently virulent virus*

Clone no.	Bud no.	Date inoculated	Virus source	Appearance* of budling 2. vii. 46	Effect on indicator plants		
					Mild	Inter-mediate	Severe
62	6	ii. 44	AR15	Good	5	0	0
64	3	ii. 44	AR15	Good	5	1	0
116	6	v. 44	AR15	Good	3	0	0
142D	8	ii. 44	AR15	Poor	0	3	3
146	6	viii. 44	AR15	Poor	0	2	4
146	8	v. 44	AR15	Fair	5	1	0
164B	4	viii. 44	AR15	Fair	4	2	0
164B	7	viii. 44	AR15	Poor	0	3	3
169B	2	ii. 44	AR15	Fair	3	0	0
169B	3	viii. 44	AR15	Poor	6	0	0
185	5	ii. 44	AR15	Poor	2	2	2
93	1	viii. 44	AR15	Poor	0	0	6
93	4	viii. 44	AR15	Good	5	1	0
93	7	viii. 44	AR15	Fair	1	0	5
104	4	viii. 44	AR15	Poor	0	0	6
AR4	4	viii. 43	AR15	Poor	0	1	5
C20†	1	vii. 43	AR15	Excellent	4	2	0
C55	2	iv. 43	AR15	Excellent	6	0	0
C5	4	xii. 42	AR4	Excellent	5	0	0
66	2	xii. 42	AR4	Excellent	1	1	4
130	1	—	Natural	Good	6	0	0
142*c*	5	—	Natural	Excellent	6	0	0

* Appearance described before indexing, using subjective standards of size, vigour and symptoms.
† The three C clones are Trinitario, the rest West African Amelonado.

did not always contain the same virus strain. Different strains were frequently obtained from the same plant, but often in proportions which reflected the condition of the host plant. In this respect, it should be noted how mild and severe strains were obtained in different proportions from the three members of clone 93.

These results do not support the conjecture that virus virulence can be altered by an individual host plant, much less by a clone. They demonstrate the complex nature of virus 1 A and how a host showing virulent-strain symptoms may nevertheless carry mild strains in easily transmissible form (cf. budlings 169B/3 and 185/5).

(2) *Direct testing*. The following experiment was done to determine whether a change in virus virulence takes place in tolerant clones and, if so, at what stage in the course of infection.

Two tolerant clones were chosen, both of Trinitario type, and propagated as rooted cuttings. Rooted cuttings of a susceptible Amelonado clone and ordinary 1-year-old Amelonado seedlings were used as controls. Two replications were set up, the first with twenty plants to each clonal or seedling set, and the second with fifteen. The plants were graft-infected from seedlings severely affected with virus 1A; a different source of virus was used for each replication.

Virus-indexing on year-old Amelonado seedlings was carried out at the following stages of infection of the experimental plants:

(1) The virus incubation period, before the plants had produced their first growth flush after inoculation;

(2) the incubation period, after the appearance of a symptomless flush (in first replication only);

(3) after the first symptom-bearing flush following inoculation;

(4) after the second symptom-bearing flush following inoculation;

(5) 12–14 months after inoculation.

Two plants per replication were indexed at each stage except the fifth, when up to eight were used. A close series of bark patches was taken from the same side of the stem or branches as that containing the infecting graft; the number of bark patches taken from each plant, and hence the number of Amelonado seedlings inoculated, varied between five and twenty-five, depending on the size of the plant and the complexity of its branching system.

In both replications the tolerance of the Trinitario clones relative to that of Amelonado was well marked; the Amelonado seedlings, perhaps because of their better root system, were more tolerant than Amelonado cuttings. In the second replication, the aggregate numbers of growth flushes produced in one year by the fifteen cuttings of C85, C73 (the Trinitario clones) and the susceptible Amelonado clone and the Amelonado seedlings were 76, 63, 32 and 62 respectively. The differences between adjacent totals were significant at the 5 % probability level. Most of the Amelonado cuttings died within the year, whereas the C85 cuttings continued to produce flushes of large leaves without distortion or necrosis, which were common with the Amelonado seedlings. Leaf symptoms occurred on most flushes of the C85 plants, but were mostly confined to the lower leaves of the flushes and were often perceptible only by transmitted light. With C73, growth was retarded, but there was little defoliation and leaf symptoms were less conspicuous than with the controls.

Despite these differences in response, there was no consistent change in virus virulence. Out of 425 test inoculations made at stages 3–5, 409 resulted in transmission of virulent virus and four of mild; twelve successful grafts failed to transmit any virus. Of 152 grafts made during the incubation period, eighty-nine transmitted virulent virus, two transmitted mild virus and sixty-one failed to cause infection. The mild strains were transmitted synchronously with virulent virus from three cuttings of C73 and one of the Amelonado clone. In addition, one cutting of C85

yielded only mild virus in an early test, but subsequently gave only virulent virus.

In this experiment, then, the transmission of mild virus appeared to be entirely fortuitous and was relatively too infrequent and sporadic to lend support to any theory postulating appreciable host effect on the virus.

(4) *Recapitulation*

Most of the trees selected for virus resistance or tolerance proved to be highly susceptible. About 10 % of the field selections were shown to resist superinfection with virulent strains of virus 1A because they were already infected with mild strains. Some of these mild strains had no readily appreciable effect on their hosts. There was no indication that the emergence of mild strains from the virus 1A complex was induced by particular host genotypes. This, together with the fact that protective mild strains can be transmitted apparently to any cacao plant, rendered worthless the selections naturally infected with mild strains, since better productivity could be expected from high-yielding selections protected in this manner.

Tolerance of virulent strains was not discovered in any Amelonado clone, but was occasionally met with in Trinitario cacao (cf. C85, C73). Such tolerance was only relative to Amelonado susceptibility and was of no economic significance.

A variable response to testing was encountered in some clones. In a few clones this resulted from slight, doubtless non-inherent, differences in the tolerance of individual budlings, but was usually ascribable to virus variation. The variability of virus 1A was found to be great.

No clone was found with resistance to virus introduced by grafting. Occasional failure of infection from successful bark grafts could be attributed to the scions being virus-free as a consequence of incomplete systemic infection of the parent plant.

Second period: testing for resistance to infection by vectors

The work of the first period was concluded in 1947 when new techniques were available and a variety of recently acquired cacao types awaited investigation for resistance. The greatest advance in technique was the adoption of insects instead of grafts for virus transmission; the latter method virtually ensures infection and so may preclude the discovery of individuals possessing merely a field resistance, as against a basic resistance to virus. Another new technique, in which the cacao embryo is used as an experimental plant (Posnette & Strickland, 1948), enabled large samples to be tested at the bean stage for comparative resistance to infection. With regard to material, most of the Trinitario and other early selections (Posnette, 1943) had become available as rooted cuttings, while seedlings of a wide range of cacao types, particularly of the Upper Amazons, were just coming into bearing, so providing beans which could be tested at that stage.

(1) *Tests using rooted cuttings*

When it had been shown that a rate of thirty infective nymphs of *Pseudococcus njalensis* Laing per plant would result in nearly 100 % infection of young cacao seedlings (Posnette & Robertson, 1950), a trial was commenced to compare the rates of infection in twenty-eight clones grown as rooted cuttings. Twenty-four of the clones were of Trinitario type and four were of West African Amelonado; all were developed from high-yielding trees. Twenty plants of each clone were used, and the order of insect transference so arranged that virus-source plants were distributed evenly among the clones. A single strain of virus 1 A in young cacao seedlings was used, and insects transferred at a rate of thirty nymphs to each test plant.

TABLE 3. *Further comparison of clones which, from a previous experiment, appeared to differ in virus susceptibility*

Low-rate group		High-rate group	
Clone	No. infected out of 20	Clone	No. infected out of 20
A12	10	A196	17
D70	13	H21	14
K31	13	O2A	16
SC1	16	S33	17
Y45	18	X46	12

The rate of infection in the sets of cuttings ranged from 2/20 to 15/20 with a mean of $8{\cdot}70 \pm 0{\cdot}69$. The five clones which gave the lowest rate (2–5/20) were then compared with the five which gave the highest rate (14–15/20) of infection in the first experiment. As is shown in Table 3, 70/100 plants became infected in the low-rate group as against 76/100 in the high-rate group, the difference being not significant.

A further twenty-four Trinitario clones were tested in the same way together with six Amelonado clones and seedlings of *Theobroma bicolor* Humb. & Bonp. and *T.* (*Herrania*) *mariae* Schumann. None of the twenty *T. mariae* seedlings, and only four of the *T. bicolor* seedlings became infected; the infection rate in the cacao clones was higher than in the first experiment, ranging from 8/20 to 20/20 with a mean of $12{\cdot}8 \pm 0{\cdot}51$.

No Trinitario clone was regarded as being sufficiently resistant to infection to merit further investigation.

The resistance of *T. mariae* is noteworthy; in several other experiments seedlings of this species and of the closely related *T. balaoense* were immune. The *Herrania* section of the genus *Theobroma* may prove useful in breeding for resistance to cacao viruses.

(2) *Tests using beans and young seedlings during the* 1948–9 *season*

Attention was now turned to the collection of seedling Amazon, Criollo and Trinitario types introduced in 1944 (Posnette, 1951).

The first tests were done using the bean-infection technique, which enabled a large number of progenies to be tested quickly for gross differences in resistance. In each test, one or more sets of twenty beans, each set from one introduced tree, was compared with a control set of twenty Amelonado beans. A virulent strain of virus 1A was used which had been transmitted several times by vectors and was thought to be relatively free of mild-strain contaminants; where it was necessary to use more than one virus-source plant, the vectors from each were distributed equally among the different cacao beans, and the order of bean infestation was such that the twenty of any one parentage could be treated as five replications each of four plants. Ten insects (*Pseudococcus njalensis*) were transferred from virus 1A-infected seedlings to each bean and allowed to feed for 24 hr. The seedlings which developed from the infested beans were observed for symptoms through at least three growth flushes.

Table 4. *Comparative resistance of beans from Parinari × Nanay trees*

Number of infected plants out of 20; vector infestation rate, 10 per bean.

Test no.	Amelonado	Mean of Parinari × Nanay
1	8	5
2	9	5
3	10	4
4	13	7
5	7	0
6	7	2
7	7	4
Mean	8·71	3·86

$n_1 = 1$, $n_2 = 6$, $F = 34{\cdot}58$, sign. $P = 0{\cdot}01$. (Analysis of variance with angular transformation of percentage transmission rates.)

Only cacao of the Upper Amazon group consistently showed any comparative resistance in these tests. Particular attention had been paid to the progeny of the vigorous Parinari × Nanay trees resulting from hand-pollination done in Trinidad (Posnette, 1945), and the results of the relevant tests are given in Table 4; here the infection rates obtained with Amelonado controls are compared with mean infection rates for sets of Parinari × Nanay progenies when more than one set had been compared with each control set.

This degree of resistance was not encountered in tests with young plants belonging to the same sets. Seedlings remaining uninfected as a result of the bean-infection tests were retested when about 6 months old, by infestation with infective *P. njalensis* at a rate of 30 insects per plant. Seven sets of ten Amelonado and ten Parinari × Nanay seedlings were compared. The resultant mean rate of infection in the introduced cacao was $3{\cdot}64 \pm 0{\cdot}61$ out of ten, and in the control $5{\cdot}43 \pm 0{\cdot}81$ out of ten; the mean difference was found to be not significant at the 5 % level of probability.

(3) *Tests using beans and young seedlings during the* 1949–50 *season*

During the 1949–50 harvest season, pods were taken from trees derived from each Upper Amazon progeny introduced in 1944 and the beans tested for resistance. The trees had been planted in type blocks of several square plots, each comprising sixteen trees; the pods were taken wherever possible from the four centre trees of the respective plots. The testing procedure was similar to that of the previous season. The beans from each pod were the subject of one test, and all the tests with pods from a family of sibling trees comprised a set. All the beans from each selected pod were tested against beans from a pod of West African Amelonado; selected and control beans were infested alternately, the rate per bean being five nymphs of

TABLE 5. *Comparative resistance of various Upper Amazon progenies.* (*Beans infested at a rate of* 5 P. njalensis *nymphs per unit*)

Upper Amazon parentage			No. of tests	Mean transmission rate (%)		S.E. diff.* between means	t*	Significant at $P=$
Parent type	Selection no.	Grand-parentage		Amazon	Amelonado			
Parinari × Nanay	63	PA 35 × NA 32	30	21·2	36·2	1·56	6·14	0·01
Parinari × Nanay	60	PA 7 × NA 32	5	31·8	65·1	4·56	4·27	0·05
Parinari × Nanay	76	PA 35 × NA 31	4	27·9	49·8	1·64	7·93	0·01
Nanay × Parinari	79	NA 32 × PA 7	6	21·1	43·0	5·02	2·72	0·05
Nanay × Parinari	82	NA 32 × PA 35	7	32·1	57·2	4·77	3·07	0·05
Parinari × ?	30	PA 103 × ?	5	31·2	42·2	4·34	1·50	—
Parinari × Parinari	86	PA 35 × PA 7	6	42·4	58·8	4·34	2·17	—
Nanay × Nanay	62	NA 33 × NA 34	5	16·8	48·2	4·16	4·77	0·01
Nanay × Nanay	92	NA 32 × NA 31	8	20·8	59·1	5·72	4·04	0·01
Scavina × ?	12	SCA 12 × ?	8	38·3	58·8	4·09	2·89	0·05
Iquitos × ?	17	IMC 53 × ?	6	34·6	41·8	3·24	1·30	—
Nanay × Iquitos	72	NA 32 × IMC 60	6	24·1	59·1	5·79	3·60	0·05
Nanay × Iquitos	73	NA 33 × IMC 60	6	24·0	41·1	1·76	5·98	0·01
Iquitos × Nanay	85	IMC 60 × NA 34	5	26·2	67·1	5·69	4·26	0·05

* Figures given in angular values.

P. njalensis from the same virus-source plant. The infested beans were planted on the following day and allowed to develop to the stage of third or fourth flush seedlings before final symptom observations were made. During the season, almost 9000 plants were used. A sample of about six randomly selected symptom-bearing seedlings, both of selected and control types, was usually retained from each test for further observation and experiment.

Cacao of Upper Amazon origin was again found to be more resistant than West African Amelonado. The results are summarized in Table 5, where the mean percentage transmission rates for each of the tests are given. The results of each set, which comprised a number of paired Upper Amazon and Amelonado percentage transmission rates, were analysed separately, using 'Student's' method for paired varieties, after transformation to the appropriate angular values.

Resistance was found to be significantly greater at the 1 % level in beans of the

Nanay type, at the 5 % level in the Scavina × ?, Nanay × Parinari and Iquitos × Nanay types, and at both levels in the Parinari × Nanay and Nanay × Iquitos types. No significant resistance was discovered in beans of Parinari × Parinari, Parinari × ? or Iquitos × ? origin. The different sets of results are not directly comparable, but it would seem that effective resistance may be resident in the Nanay population.

The general level of comparative resistance shown in these tests is not considered to be of immediate practical value, but further work is being done to seek effective resistance or immunity among the progenies which so far have shown most promise. The method is eliminative, involving the testing and retesting of seedlings which remain uninfected after a heavy bombardment with infective vectors at the bean stage.

Table 6. *Comparative resistance of various Upper Amazon progenies. Beans infested at a rate of* 30 P. njalensis *nymphs per unit.*

Upper Amazon parentage		No. of tests	Mean transmission rate (%)		S.E. diff.* between means	t*	Significant at $P=$
Parent type	Selection no.		Amazon	Amelonado			
Parinari × Nanay	63	7	66·7	91·8	6·49	2·88	0·05
Nanay × Parinari	79	9	63·7	92·5	3·79	5·57	0·01
Nanay × Parinari	82	5	78·0	92·8	5·78	2·16	—
Parinari × Parinari	86	3	86·0	80·6	—	—	—
Nanay × Nanay	62	8	42·7	84·7	4·73	5·54	0·01
Nanay × Nanay	92	20	63·8	89·6	3·01	6·07	0·01

* Figures given in angular values.

The comparative resistance of Upper Amazon seedlings has not yet been determined, but significant degrees of resistance were encountered when beans of this type were infested at a rate of 30 infective nymphs of *P. njalensis* per unit, which is considered to be about the optimum for infection of West African Amelonado beans (Posnette & Robertson, 1950). The results of these tests, which were carried out in the manner already described in this section, are given in Table 6.

As a result of the higher vector infestation rate, the mean percentage transmission rates were considerably increased, but except for one Nanay × Parinari progeny and the Parinari × Parinari progeny, the Upper Amazon beans were significantly more resistant to infection than the controls. On the whole the Nanay progenies again seemed most promising. In marked contrast, Parinari beans appear to be no more resistant to virus infection than West African Amelonado.

Upper Amazon cacao is not only more resistant to infection than Amelonado, but is also more tolerant. This greater tolerance is expressed in two ways: (1) by a longer virus incubation period (possibly an indication of greater virus resistance *after* infection) and (2) by a milder and sometimes evanescent symptom expression. Table 7 demonstrates the greater tolerance of the Upper Amazon seedlings by these two criteria. The Upper Amazon and Amelonado seedlings compared in

Table 7 were selected arbitrarily from those infected in the appropriate tests. Two sets of seedlings are cited in respect of each parental group, except with selection 92, where three are given, the first set being duplicated. The two sets are given merely as a range; the first pertains to a test in which the comparative resistance of the Upper Amazon beans was relatively high, and the second to one in which resistance was lower.

TABLE 7. *The tolerance of Upper Amazon seedlings compared with Amelonado seedlings, when infected at the bean stage*

Upper Amazon parentage			No. pairs Amazon and Amelonado seedlings compared	Incubation* period		No. seedlings with symptoms			
						Mild		Severe	
Parent type	Parent progeny no.	Mother tree no.		Amaz.	Amel.	Amaz.	Amel.	Amaz.	Amel.
Parinari × Nanay	63	883	6	3·00	2·17	3	0	3	6
Parinari × Nanay	63	882	9	1·56	1·33	5	1	4	8
Parinari × Nanay	60	973	5	1·80	2·20	3	0	2	5
Parinari × Nanay	60	962	6	2·50	1·83	0	0	6	6
Nanay × Parinari	79	1151	5	2·00	1·60	1	0	4	5
Nanay × Parinari	79	414	5	3·00	2·40	2	0	3	5
Nanay × Parinari	82	666	6	2·33	1·50	1	0	5	6
Nanay × Parinari	82	666	6	2·33	1·17	1	0	5	6
Parinari × ?	30	606	6	2·33	1·67	4	0	2	6
Parinari × ?	30	539	6	3·33	2·33	3	0	3	6
Parinari × Parinari	86	1043	5	4·00	1·80	3	0	2	5
Parinari × Parinari	86	955	6	2·67	1·83	2	0	4	6
Nanay × Nanay	92	880	2	3·50	1·00	1	0	1	2
Nanay × Nanay	92	791	2	2·00	1·00	2	0	0	2
Nanay × Nanay	92	795	5	1·80	1·60	2	0	3	5
Scavina × ?	12	27	6	1·83	1·17	2	0	4	6
Scavina × ?	12	27	10	2·00	1·10	4	0	6	10
Iquitos × ?	17	524	4	3·25	1·50	0	1	4	3
Iquitos × ?	17	1131	6	1·83	1·17	0	0	6	6
Nanay × Iquitos	72	1523	5	2·60	1·80	2	0	3	5
Nanay × Iquitos	72	1470	4	2·50	1·75	1	0	3	4
				Total		42	2	73	113

* Figures refer to the particular growth flush (first, second, etc.) on which the first symptoms appeared; the fractional values result from averaging.

Experiments are being conducted to investigate the comparative availability to vectors of the virus in tolerant Upper Amazon seedlings. Virus tolerance, *per se*, would not be considered a desirable property of cacao, unless associated with resistance or reduced virus availability and consequently a slower rate of spread. The results of only one test are as yet available, and they indicate that tolerance does not presume either reduced availability of virus to vectors or any qualitative virus change.

DISCUSSION

The literature on resistance to virus diseases of trees is scanty; of all the diseases described by Hildebrand, Berkeley & Cation (1942) only buckskin disease of cherries is controllable by resistance, and in that case the rootstock species is

concerned (Rawlins & Parker, 1934). Bond (1944) has reported tolerance to phloem necrosis in varieties of tea. van der Plank (1949*a*) has suggested that most trees resist virus diseases, and quoted cacao as an exception. To us it seems that virus infection of tree species is more common than the absence of obvious symptoms would suggest, but tolerance and resistance, including the tendency for both to increase with age, prevents many viruses from causing diseases of great economic importance.

Before discussing the use of resistant cacao in West Africa, we must consider the background of the problem. There is a tradition that almost all the cacao of the Gold Coast is descended from a few seeds introduced from Fernando Po in 1879. Although this is not strictly true, since a large quantity of seed has been distributed from the Aburi cacao that was introduced from San Thomé about 1890, there is no doubt that the bulk of Gold Coast cacao is descended from these early introductions. The Amelonado type is remarkably uniform in habit, botanical characters and reaction to disease, as well as in commercial produce, but that this is characteristic of the type, and not a result of its immediate origin, is shown by the equal uniformity of Amelonado cacao in Nigeria and the Ivory Coast.

The pattern of cacao cultivation varies little throughout the country; the established 'farm' consists of a few acres of closely set trees (averaging about 600 per acre), the branches of which interlace to form a continuous canopy. When a cacao farm is planted, many of the original forest trees are left to provide a stratified broken shade which seems to be particularly suited to cacao. Though the average farm is small, the conception (van der Plank, 1949*b*) that each is more or less discrete is false. In 'cacao country', hundreds of acres, including a great many farms, may be under an almost continuous cacao canopy, the individual farm boundaries being unmarked or indicated only by occasional ground plants. Thus cacao is grown in an almost closed forest in which the lowest tier of tree growth is occupied by cacao trees. This pattern is not followed in the drier regions of the Gold Coast and Nigeria, however, where cacao is grown with little or no shade.

It seems probable that viruses spread to cacao from a few widespread forest tree species, and outbreaks are now frequent though markedly discontinuous throughout West Africa between Liberia and the Cameroons. Most outbreak areas are relatively small, and virus transmission from the indigenous reservoirs to cacao seems to be rare and sporadic. This is borne out by experiment; virus tends to be of exceedingly low availability to vectors in almost all known alternative host species. When the virus is transferred to cacao, however, its availability is greatly enhanced and rapid spread of the disease ensues. This has occurred most spectacularly in the Eastern Province of the Gold Coast, previously the zone of greatest production.

Such, then, is the epidemiological framework. The primary aim in disease control is, and must continue to be, the arrest of rapid spread of virus in the remaining healthy cacao. The secondary aim is to rehabilitate the devastated areas with improved types of cacao. As it is virtually impossible at present to distinguish

healthy from infected trees of most alternative host species, control of the latter must be incomplete, and so recurrence of swollen-shoot disease in new plantings can be expected. The desirability of limiting spread of disease in such new plantations is self-evident. How can the properties of tolerance, protection and resistance to infection best be utilized to these ends?

Until such time as it may seem probable that the remaining cacao trees in the Gold Coast cannot be saved, tolerant varieties which would harbour virulent virus without showing conspicuous symptoms would be a grave menace. There seems little to recommend the development of even a highly tolerant type unless one attribute of the tolerance were a greatly reduced availability of virus to vectors. Tolerance is relative, and although we have found some Trinitario clones and Upper Amazon seedlings significantly more tolerant than the susceptible Amelonado type, it seems unlikely at present that they will reach the standards required for commercial planting. No symptomless carrier of severe virus 1A has been encountered.

The use of mild viruses to protect plants against virulent strains is not new. It was suggested by Johnson (1937), and the subject has been reviewed by Price (1940). Recently, Grant & Costa (1951) have found a mild strain of the tristeza virus and discussed its possible use in the control of this citrus disease. Early experiments with cacao viruses failed to show adequate protection (Crowdy & Posnette, 1947), because the viruses used were not closely related. Now mild strains of virus 1A have been found which give virtually complete protection against virulent strains. Because no tested cacao plant has been found preferentially susceptible to mild strains of the complex, as is said to occur with sugar-beet and the curly-top virus (Giddings, 1950), and because apparently any cacao plant can be infected with these mild strains, it seems unlikely that the selected 'survivors' with mild-strain infection possessed any intrinsic value.

Although protection is the only form of quasi-resistance which could be applied to the existing cacao population, its immediate adoption seems undesirable mainly because the long-term effects of mild-strain infection on vigour and yield are not yet known; and also because the danger of a change in virulence from mild to severe cannot be ignored. A further objection is that mild strains of virus 1A protect against other strains of that virus only and not against the other at present less important cacao viruses. Indeed, the combination of some other viruses with the mild virus 1A in protected plants might have severe effects.

Despite these objections, special circumstances may arise in which the protection method has to be used. Should control by eradication become impracticable in local areas, virus spread would, it is thought, be impeded by 'protecting' a belt of trees on the periphery of swollen-shoot outbreaks.

Resistance in the strict sense, that is, resistance to primary infection by vectors, has been found only in Upper Amazon types, where it is frequently associated with tolerance, and in *Herrania* species. Further work should be directed towards the

development of this type of resistance which has the widest application in the rehabilitation of devastated areas. Up to now we have done no more than develop a workable technique and demonstrate that resistance to infection occurs within the Upper Amazon population, possibly in the Nanay type in particular. Further testing and selection must be done before the extent of this resistance can be judged. The range of Amazon material introduced to the Gold Coast was limited by the need to extend the collection to all available types of cacao in the hope of finding a resistant type. Now that we know where to look, the introduction of more Nanay material might yield genotypes nearer to the immune ideal.

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STUDIES IN *RUBUS* VIRUS DISEASES

I. A LATENT VIRUS OF NORFOLK GIANT RASPBERRY

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(With Plate 23)

A selection of Norfolk Giant raspberry is infected with a virus transmissible by *Amphorophora rubi* Kalt. after short feeding periods on infected plants and persisting for at least 18½ hr. in the aphid. This virus is identified with one which is carried without symptoms by Norfolk Giant and Baumforth's Seedling B, and causes necrosis on *Rubus henryi* and mosaic symptoms on *R. saxatilis*, American black raspberry *R. occidentalis* (var. Cumberland) and the red raspberry varieties Chartham, Malling Landmark and St Walfried. The virus is present in some commercial stocks of Baumforth's Seedling B, Burnetholm Seedling, and the Malling varieties Enterprise, Notable and Promise. The name raspberry leaf mottle is proposed.

Introduction

An investigation into the causes of failure of vigour and cropping of raspberries in Scotland was initiated by the Agricultural Research Council in November 1943 at the request of the, then, Agricultural Improvement Council for Scotland. The Scottish raspberry industry was experiencing a serious decline following a succession of unfavourable seasons, the failure in vigour and cropping of the staple variety, Lloyd George, and the outbreak of a lethal leaf-curling disease in the substitute variety, Norfolk Giant.

Leaf curl was early shown to be caused by a virus (Harris, Bryce & Foister, 1943), and it was clear that the core of the general problem of raspberry decline was the unthriftiness of the stocks of planting cane due to the effects of cumulative virus infection. This led to an inquiry into the virus diseases affecting raspberries in Scotland, in continuity with the work already begun by Harris and Prentice at East Malling, and to the development of methods of selecting and perpetuating the healthy stocks of planting cane urgently needed by the industry.

These essentially complementary programmes of work have run side by side since 1944. The problem of virus-induced decline of raspberries has proved more complex than anticipated, but progress has been made towards a solution.

Harris (1940) separated the symptoms of mosaic diseases of the European red raspberry into two, mosaic 1 and mosaic 2. The first of these, characterized by a diffuse and conspicuous chlorotic leaf pattern, was thought to be caused by one

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virus. The mosaic 2 group, characterized by a leaf pattern of sharply defined, angular and incised chlorotic spots, comprised two or more diseases of differing intensity. In addition to this qualitative difference, mosaic 2 diseases were found to be expressed fully only by the variety Baumforth's Seedling B, whereas certain varieties, such as Lloyd George, behaved as symptomless carriers of these diseases. The differential reaction of Baumforth's B was established by Harris (1933) and led to the choice of this variety as an indicator for the detection of virus infection in, so-called, tolerant varieties. It was later found that Norfolk Giant also reacted to viruses causing mosaic 1 and 2 diseases in much the same way as Baumforth's B (Harris, 1944; Prentice & Harris, 1950). On this account and because samples of the East Malling mosaic-free (New Zealand) clone of Norfolk Giant (L234), when grafted to healthy plants of Baumforth's B, had produced no symptoms on the latter, it was assumed that the L234 clone of Norfolk Giant was virus-free (Prentice & Harris, 1950). Therefore, since 1944, this clone was used at Dundee as an indicator in stock-testing work.

Evidence that this assumption was incorrect was provided by two experiments carried out in 1948. A set of *Rubus saxatilis* plants to which aphids, *Amphorophora rubi* Kalt., were transferred from 'virus-free' plants of L234 Norfolk Giant developed conspicuous mosaic symptoms. Later the same year, five plants of another test plant, *Rubus henryi*, developed necrosis after being grafted with L234 scions. At East Malling, in 1947, it had also been found that plants of the raspberry variety Chartham became severely dwarfed when grafted to L234 Norfolk Giant (Prentice, private communication). The presence of a hitherto undetected virus or viruses in the L234 clone implied by these results has been confirmed by the more recent work which forms the subject of this paper. This virus in the L234 clone has proved to be transmissible by *Amphorophora rubi* and is identified with the cause of visible mosaic symptoms on a number of varieties of raspberry.

Transmission by grafting

Method

Experiments by both British and American workers have shown that viruses infecting raspberries are not transmissible by sap inoculation either to raspberries or to a range of solanaceous and other hosts (Harris, 1933; Bennett, 1927). Harris (1933) showed that inarch grafting was an efficient method of artificial inoculation. This method proved feasible at Dundee but made extravagant demands on material and space and involved considerable delay, as indicator plants rarely showed diagnostic symptoms in the current year. After experimentation with alternative methods in 1945, a modification of Harris's method according to the standard horticultural technique of bottle grafting (Garner, 1947) was adopted, and this has since been used almost exclusively. The essential details of this method are as follows. Scions, which must be the topmost portions of young, growing canes, of

convenient length (4–8 in.), are prepared for grafting by trimming off all but the young, newly expanded, tip leaves. Young canes on the stock plants, grown in pots, have their topmost portions similarly trimmed. The scion is then grafted to the stock as in Harris's inarch method (1940); the graft is held firmly in position and the junction bound over with a strip of adhesive tape. A proprietary brand of adhesive plastic (PVC) tape has recently been found very suitable. The grafted cane is then tied to a stake and a 6 × 1 in. boiling tube wired on to the latter in such a position that the cut end of the scion is submerged 2–3 in. when the tube is filled with water (Pl. 23, fig. 1). Under optimum conditions, stock and scion unite in 14–20 days. Nutrient solutions have not proved to be more efficient than tap water in maintaining the scion, but the tube must be kept filled with water until union has occurred.

Rapid development of symptoms can be induced by pinching out the growing point of the grafted cane 8–10 days after grafting and encouraging the development of side shoots immediately below the point of union. On occasions, symptoms have appeared on such shoots 14 days after grafting. Under cool, well-ventilated, glasshouse conditions and without any supplementary treatment, this method of grafting has proved highly successful. Grafting can be carried out over the entire growing season, from the time the young canes on stock plants are large enough to handle (usually late April or early May in Scotland), until growth ceases in late August or early September. Bottle grafting cannot be done under outdoor conditions, but grafted plants can be transferred to an outdoor plunging ground, once satisfactory union has occurred, and grown on for further observation.

The advantages of this technique are (1) an economy of infector material, as many scions can be cut from a single plant, and (2) an ensurance of positive graft unions, as, under glass, the unions can readily be inspected and individual stock plants regrafted until a satisfactory union is obtained.

Unless otherwise stated, all references to grafting in the following account imply bottle grafting. The scion (and its plant of origin) will be referred to as the infector and the stock plant as the indicator. Grafting was carried out under ordinary glasshouse conditions, where aphid infestation was controlled by routine nicotine fumigations.

Results

The data from grafting experiments carried out in the years 1947–50 are presented below and summarized in Table 1.

Rubus *species*

Experiments in 1947 with plants of a number of *Rubus* species,* showed that *R. henryi* became necrotic when grafted with L 234 Norfolk Giant. Plants grafted with scions from seedlings raised in that year from seeds of Norfolk Giant showed no symptoms. The experiment was repeated in 1949. No symptoms developed

* We are indebted to Sir Edward Salisbury, Director, Royal Botanic Gardens, Kew, for this material.

on five plants of *R. henryi* grafted with seedling raspberry scions in May; but five plants grafted with L234 Norfolk Giant on 22 May all developed necrosis by 16 June, in just over 3 weeks from the date of grafting.

Experiments with *R. saxatilis* in 1945 and 1947 showed that this species expressed symptoms when infected with a number of distinct viruses. The plants used in these and subsequent experiments were raised from runners collected in the field. In July 1948, five plants were grafted to potted plants of L234 Norfolk Giant.

TABLE 1. *Results of graft transmission of leaf mottle*

Indicator	Infector	Year of graft	No. of plants	Necrosis	Mosaic	No symptoms
R. henryi	L234	1947	3	3	—	—
	L234	1949	5	5	—	—
	Virus free seedling	1947	2	—	—	2
	Virus free seedling	1949	5	—	—	5
	R. saxatilis (1948 exp.)	1949	5	5	—	—
	R. saxatilis (healthy)	1949	5	—	—	5
R. saxatilis	L234	1948	5	—	5	—
	Baumforth's B	1947	5	—	1	4
	Baumforth's B	1948	2	—	2	—
	Ungrafted controls	1948	5	—	—	5
R. occidentalis var. Cumberland	L234	1949	5	5	—	—
	Ungrafted controls	1949	3	—	—	3
Chartham	L234	1949	5	—	5	—
	Virus-free seedling	1949	5	—	—	5
	Ungrafted controls	1949	5	—	—	5
Lloyd George	L234	1949	5	—	—	?5
	Ungrafted controls	1949	5	—	—	5
Malling Landmark	L234	1949	5	—	5	—
	Ungrafted controls	1949	5	—	—	5
	Baumforth's B	1950	2	—	1	1
	Burnetholm Seedling	1950	5	—	5	—
	Malling Enterprise	1950	1	—	1	—
	Malling Notable	1950	1	—	1	—
	Malling Promise	1950	2	—	2	—
St Walfried	L234	1949	5	—	5	—
	Ungrafted controls	1949	5	—	—	5

Four of the *R. saxatilis* plants showed conspicuous interveinal chlorotic patches in November 1948, and again in the following spring; the fifth plant died during the winter (Pl. 23, fig. 2). When the four surviving plants were grafted, in April 1949, to plants of *R. henryi*, all the latter developed necrosis within a month. Five healthy control plants of *R. saxatilis* were grafted to *R. henryi* at the same date, but no symptoms developed on either indicator or infector plants though the unions were quite sound.

Five plants of *R. occidentalis* var. Cumberland, raised from rooted tips imported from the U.S.A. from a stock reputed to be mosaic-free, were grafted with L234 Norfolk Giant scions in April and May 1949. All five developed necrosis of the

cane tips, two of them in 3 weeks. Shoots produced after this initial reaction bore slightly malformed and distorted leaves with conspicuous chlorotic spots concentrated round the main veins (Pl. 23, fig. 3). In 1950, similar chronic symptoms again showed on two of these plants retained in the glasshouse until July. Three ungrafted control plants showed no visible symptoms throughout this period.

Raspberry varieties

Chartham. In the course of routine testing work at East Malling in 1946, Prentice (private communication) found that five plants of the variety Chartham, inarch grafted outdoors to five plants of L234 Norfolk Giant, showed severe mosaic and stunting of canes in 1947. The five grafted plants of L234 Norfolk Giant and five ungrafted plants of Chartham showed no symptoms.

In 1949, at Dundee, five plants of Chartham grafted with L234 Norfolk Giant developed mosaic symptoms, accompanied by leaf distortion and stunting of growth. By April 1950, one plant was dead; the fruiting canes on the other four plants produced a few distorted laterals and the young canes were dwarfed and showed severe mosaic symptoms. No symptoms developed in 1949 on five plants grafted with scions from current year seedling raspberries or on five ungrafted control plants. In 1950, however, three of the former showed slight interveinal chlorosis, and scions from two of these induced necrosis on *R. henryi* plants to which they were grafted in July 1950. This result is believed to be due to a virus initially present in the indicator plants and not to virus infection of the seedling raspberry scions. The Chartham plants were not indexed for virus content before use in this experiment, but other plants of the same stock were found to be infected with an unidentified virus that produced necrosis on *R. henryi.*

Malling Landmark. In earlier experiments it was noticed that whenever plants of Malling Landmark were grafted to a mosaic-infected Norfolk Giant, the former always developed a more complex symptom picture than could be accounted for either by the mosaic virus known to be present in the infector or by the results of check grafts from the infected Landmark to L234 Norfolk Giant. The constant component of the complex was a chlorotic blotching, resembling mosaic 2 disease, that had been found widely distributed in commercial stocks of Malling Landmark. This symptom is distinct from the faint mosaic mottle referred to by Prentice & Harris (1950). The same authors also showed that Malling Landmark is a symptomless carrier of the causes of mosaic 2 diseases.

As part of a programme of nuclear stock propagation in 1947, twenty-five symptom-free plants of Malling Landmark were selected from a carefully rogued commercial stock. Scions from the selected plants were grafted to L234 Norfolk Giant in the same year. None of the latter developed visible symptoms, but on sixteen of the Malling Landmark scions a conspicuous chlorotic blotching developed some 6 weeks after grafting. The parent plants of the nine scions that failed to show symptoms were retained for propagation in 1948, when test grafts to Norfolk Giant

were again made. Scions from all nine plants developed blotching when grafted to L234 Norfolk Giant, although no symptoms were visible either on the parent Malling Landmark plants or the L234 indicators.

The reciprocal combination of L234 scions on symptom-free plants of Malling Landmark was made in 1949. Five plants all developed chlorotic blotching a few weeks after being grafted with L234 Norfolk Giant, whilst five ungrafted control plants only showed a faint chlorotic mottle (Pl. 23, fig. 4).

Other varieties. Prentice & Harris (1950) recorded that plants from two mosaic-free selections of Norfolk Giant (L209 and L234) produced no symptoms on Baumforth's B indicators to which they were grafted. Of five Baumforth's B plants grafted at Dundee in 1948 with scions of the L234 stock, none has since showed any difference in appearance from the five ungrafted control plants.

That Baumforth's B, however, may be a symptomless carrier of the virus present in the L234 stock is suggested by the following results. One of five, and both of two plants of *R. saxatilis*, grafted in 1947 and 1948 respectively, to healthy plants of Baumforth's B showed mosaic symptoms indistinguishable from those on the plants grafted to L234 Norfolk Giant in 1948. In 1950, one of two plants of Malling Landmark was successfully grafted with a healthy Baumforth's B scion and developed chlorotic blotching four weeks after grafting.

Five St Walfried plants, known to be free from mosaic 2 and yellows diseases (Cadman & Harris, 1951), showed no symptoms in 1949 after grafting with L234 Norfolk Giant scions; in 1950, all the plants showed faint interveinal chlorotic patches on the leaves. Five similar but ungrafted control plants did not show symptoms in either year. Interveinal chlorosis appeared on three plants of St Walfried grafted with L234 scions in 1948 and also on five plants grafted with healthy Baumforth's B scions in the same year.

Some results from early experiments with Malling Seedling K suggested that it also might show symptoms when infected with the virus disease present in L234 Norfolk Giant. This was confirmed by an experiment made in 1949, in which five plants of the variety were grafted with L234 scions. None of these showed symptoms in the current year but the young canes developed conspicuous chlorotic blotching in spring 1950. These symptoms were absent from foliage produced later in the season under glasshouse conditions.

Scions from plants of Burnetholm Seedling and the Malling varieties Enterprise, Notable and Promise all induced chlorotic blotching on plants of Malling Landmark to which they were grafted in July 1950. Samples of commercial stocks of the first three of these varieties have all been found to show chlorotic leaf symptoms similar to those described above on St Walfried. Scions of a seedling raised from a cross with Malling Landmark and of a Dutch variety, Gertrudis, raised by Dr I. Rietsema, from a cross between inbred lines of Lloyd George and Pyne's Royal, have shown symptoms closely similar to those described on Malling Landmark when grafted to L234 Norfolk Giant.

Conclusions

It is concluded from these results that the L234 selection of Norfolk Giant, although free from visible mosaic, is not virus-free, and that the variety behaves as a symptomless carrier of a virus for which the name leaf mottle is proposed. It is believed that a sufficiently large number of individual plants has been sampled to warrant the conclusion that the L234 selection is entirely infected. To what extent other commercial stocks of this variety are similarly infected is unknown.

It may also be inferred that Baumforth's Seedling B is a symptomless carrier of this virus which was also present in the samples examined of Baumforth's B, Burnetholm Seedling and the Malling varieties Enterprise, Notable and Promise.

On account of the clear differences in symptomatology from other previously described virus diseases of raspberries, leaf mottle is considered etiologically distinct.

Transmission by aphids

Method

Experiments with insects as vectors of leaf mottle were limited to the two species, *Amphorophora rubi* Kalt. and *Doralis* (*Aphis*) *idaei* V.d.G. that had earlier been shown to be vectors of certain other raspberry viruses (Cadman & Hill, 1947).

The aphid stocks used were reared under glass from fundatrices collected each spring either in the field or, in more recent years, from cultures of eggs deposited the previous autumn by aphid colonies maintained outdoors in gauze-covered cages. The cultures were reared in muslin-covered sleeve cages on plants either of L234 Norfolk Giant or plants raised from seed in the current year. As there is no evidence of seed transmission of raspberry viruses, seedlings were assumed to be virus-free.

For experimental purposes, aphids were fed either on detached leaves or on caged shoots of virus-infected plants. Such source plants are referred to as the infectors and the periods for which aphids fed on the infectors as the infection feeding period (I.F.P.). After an initial I.F.P., the aphids were transferred to sets of indicator plants and allowed to feed on these for a time referred to as the test feeding period (T.F.P.). In some cases, aphids, after being given a T.F.P. on one indicator, were retransferred to a fresh indicator and subsequently again retransferred. Such serial transfers are denoted as first transfer, second transfer and so on. The number of plants infected in each experiment will be shown as a fraction, the numerator being the number of indicator plants developing symptoms and the denominator the total number of indicators receiving a particular treatment.

All indicator plants were fumigated with nicotine vapour before use and again immediately after the appropriate aphid feeding period had been completed. Thereafter, the glasshouses in which the plants were grown were given routine nicotine fumigations.

RESULTS

Preliminary experiment

From a culture of *Amphorophora rubi* reared on L234 Norfolk Giant, aphids were transferred to a plant of Lloyd George, known to be affected with a complex virus disease. After an I.F.P. of 48 hr., twenty aphids were transferred to each of five plants of *Rubus saxatilis*. On the same date, twenty aphids from the stock culture on Norfolk Giant were transferred to each of a second set of five *R. saxatilis* plants. On both sets the aphids were allowed a T.F.P. of 48 hr. The experiment was completed on 19 June, and on 5 July two plants of the first set had developed severe leaf distortion and chlorosis. One of these plants was grafted successfully to a plant of L234 Norfolk Giant, which subsequently developed mosaic 2 disease. On 10 August three more plants of the first set and two of the second (control) set showed interveinal chlorotic spots (Pl. 23, fig. 5). By April 1949, four of the five control plants showed these symptoms; the remaining plant died during the winter. No symptoms appeared on five *R. saxatilis* plants to which no aphids had

TABLE 2. *Transmission of leaf-mottle virus by* Amphorophora rubi

Year of experiment	Indicator	Aphids per plant	I.F.P. 10 min.	½ hr.	1 hr.	2 hr.	4 hr.	8 hr.	16 hr.	24 hr.	48 hr.	Control
1949	*R. saxatilis*	5	0/5	1/5	0/5	0/5	0/5	1/5	4/5	3/5	1/5	0/5
1950	*R. saxatilis*	20	—	—	0/5	0/5	0/5	4/5	4/5	—	—	0/5
1950	*R. occidentalis* var. Cumberland	20	—	—	4/5	0/5	3/5	1/5	1/5	—	—	0/5
	Total		0/5	1/5	4/15	0/15	3/15	6/15	9/15	3/5	1/5	0/15

been transferred, or on five plants, each of which received twenty *Doralis idaei* transferred from the stock culture reared on L234 Norfolk Giant. The symptoms on all four of the *Amphorophora rubi* control plants were indistinguishable from those shown by the *Rubus saxatilis* plants grafted to L234 Norfolk Giant in 1948 described above.

It was, therefore, concluded that *Amphorophora rubi* is the vector of a virus or viruses present in L234 Norfolk Giant, and further experiments were carried out in 1949 and 1950 to determine more precisely the virus/vector relations. The positive results of these experiments were meagre and, to some extent, inconsistent.

In one set of experiments, the results of which are given in Table 2, *A. rubi*, fed for periods varying from 10 min. to 48 hr. on L234 Norfolk Giant, were transferred to healthy plants of *Rubus saxatilis* and *R. occidentalis* var. Cumberland on which they were allowed a T.F.P. of 48 hr. The aphids used for the 10 min. and ½ hr. infection feeds were previously starved for 18 hr. Symptoms developed on a proportion of all the indicator plants that received aphids fed for ½ hr. or more on the infector. No symptoms developed on indicators that received aphids given a 10 min. I.F.P. or on control plants that received aphids direct from the stock cultures. Symptoms on all the infected *R. saxatilis* plants were indistinguishable

from those of leaf mottle and had an average incubation period of 27 days. Acute symptoms, consisting of necrosis of stem tips and petioles, leaf curling and conspicuous yellow blotching, developed on five of the Cumberland plants 8 days after aphids had been transferred to them; similar symptoms showed on other plants in 11–16 days (Pl. 23, fig. 6). New growth on all the infected plants subsequently showed the chronic symptoms of leaf mottle described above in the grafting experiments.

In a second set of experiments, the details and results of which are shown in Table 3, *Amphorophora rubi*, fed for lengthy periods on L 234 Norfolk Giant, were subjected to a series of transfer feeds on healthy plants of *Rubus saxatilis* and *R. occidentalis* var. Cumberland. All the plants that became infected showed symptoms indistinguishable from leaf mottle, but no symptoms developed on indicators that received aphids from the stock cultures.

TABLE 3. *Persistence of leaf-mottle virus in* Amphorophora rubi

I.F.P. indefinite and 16 hr.; T.F.P. various.

Year of experiment	Indicator	I.F.P.	Aphids* per plant	Transfer: T.F.P.:	1st ½ hr.	2nd 2 hr.	3rd 4 hr.	4th 12 hr.	5th 24 hr.
1948	*R. saxatilis*	Indefinite	20		0/5	1/5	1/5	0/5	1/5
1949	*R. saxatilis*	Indefinite	20		2/5	0/5	0/5	0/5	0/5
				T.F.P.:	10 hr.	24 hr.	24 hr.	48 hr.	—
1950	*R. occidentalis* var. Cumberland	16 hr.	20		5/5	0/5	0/5	0/5	—

* Twenty aphids were transferred from the infector to each indicator of the first transfer set. Small numbers of aphids died or were lost in the course of the subsequent transfers.

The results of these experiments are interpreted as showing that the disease transmitted by *Amphorophora rubi* was caused by only one virus, and that this persisted in the aphid for at least 18½ hr.* There are indications that vector efficiency increases with length of infection feeding period. It is presumed that this virus was the cause of the mosaic disease studied in the grafting experiments, and was probably the only virus present in the L 234 infectors. Proof of this identification has not yet been obtained.

From the results of the 1948 experiment and from more recent work, it seems unlikely that *Doralis idaei* is a vector.

DISCUSSION

Though the leaf-mottle virus appears to be widespread among commercial raspberry stocks, its significance in the field cannot yet be assessed. The grafting experiments have shown that, on the varieties Chartham, Malling Landmark and Malling Seedling K, this virus produces severe diseases and these are commonly found on commercial stocks. Varieties, such as Baumforth's B, Malling Promise and Norfolk

* *Note added in proof.* More recent experiments have shown that the leaf mottle virus rarely persists in the aphid for longer than 4 hr.

Giant, that show no leaf symptoms, appear to be unaffected; in fact, the L234 selection of Norfolk Giant is superior in vigour to other commercial stocks of this variety. However, as will be shown in a later publication, the leaf-mottle virus appears to be an important component of disease complexes affecting St Walfried and Lloyd George.

Aqueous extracts of rosaceous plants contain large amounts of tannin which acts as a protein precipitant and militates against mechanical transmission and serological study of the viruses infecting these hosts. Thus, Bennett (1927) and Bawden & Kleczkowski (1945), respectively, showed that sap from raspberry and strawberry plants inactivated tobacco mosaic virus. The latter authors also found that extracts of virus-infected strawberry leaves were protein-free and they were unable to produce antisera against such extracts. Raspberry viruses have not yet been transmitted successfully to hosts outside the genus *Rubus*, and work with this group of viruses is dependent on test transmissions to indicator plants that show diagnostic symptoms when infected. As a result, viruses may readily escape detection if they fail to produce symptoms on the test plants employed. In the present instance, the leaf-mottle virus escaped notice so long as Norfolk Giant and Baumforth's B were the only indicators used. The choice of *Rubus henryi*, *R. saxatilis* and *R. occidentalis* as supplementary indicators was, however, largely fortuitous and arose out of a search for test plants that would prove to be more susceptible to infection by means of aphids than commercial varieties of red raspberry (*R. idaeus*).

R. henryi has so far been used only in grafting experiments, and its hypersensitivity renders it useless as a differential host plant. Although no claim can justifiably be made that absence of symptoms on *R. henryi* in graft tests implies virus freedom, no interaction was observed when healthy plants of *R. saxatilis* and *R. henryi* were grafted together, and no symptoms developed on the latter when grafted with scions from seedling raspberries. No experiments to determine whether raspberry viruses are seed transmissible are known to the writer, though there is ample evidence to indicate that seed transmission is improbable.

Both *R. saxatilis* and *R. occidentalis* have proved readily infectible by means of *Amphorophora rubi*. Cadman & Hill (1947) found that the virus of mosaic 2 was transmitted to Norfolk Giant by means of large numbers of aphids. Attempts to repeat this, using small numbers (20–50) of aphids per plant, failed, and there is now evidence to show that varieties of *Rubus idaeus* are more resistant to aphid-borne infection than the two above-mentioned species (Cadman, unpublished). *R. saxatilis* is an inconvenient test plant because symptoms appear slowly and because, under glass, the plants complete their main vegetative growth very early in the year, usually before cultures of aphids are available. Neither *Amphorophora rubi* nor *Doralis idaei* have been found infesting *Rubus saxatilis* in the field, and it is evident, from study of the behaviour of *Amphorophora rubi* in the experiments described, that the plant is not a particularly favourable host for this aphid.

The extreme susceptibility of *Rubus occidentalis*, the American black raspberry,

Fig. 1.

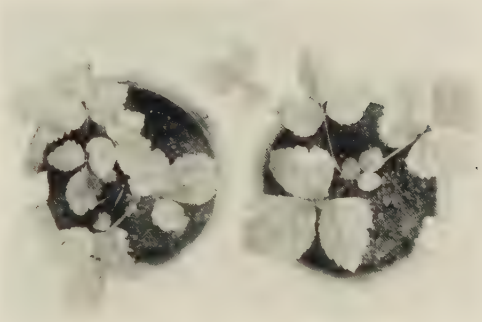

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

to infection with red raspberry virus diseases has been repeatedly affirmed by North American workers. Both the results of the above experiments and of others, to be reported elsewhere, confirm that this host may, under certain conditions, react very quickly after infection. Both the acute and chronic symptoms of leaf mottle on this host are similar to those described by Bennett (1927, 1932) on Cumberland plants with red raspberry mosaic disease. These analogies will be discussed in a later publication.

Grateful acknowledgement is made to Dr W. E. Whitehouse, U.S.D.A., for supplies of experimental material; to Dr R. V. Harris and Dr I. W. Prentice for much help and constructive criticism; and to the Agricultural Research Council who have financed the work.

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EXPLANATION OF PLATE 23

Fig. 1. Bottle-grafting technique; appearance of grafted plants 1 and 3 weeks respectively after grafting.

Fig. 2. Plants of *Rubus saxatilis*. Left, graft infected with leaf mottle virus; right, ungrafted healthy plant.

Fig. 3. Leaf from plant of *Rubus occidentalis* var. Cumberland, grafted with L 234 Norfolk Giant.

Fig. 4. Leaf from plant of Malling Landmark graft-infected with leaf-mottle virus.

Fig. 5. Leaf from plant of *Rubus saxatilis* infected with leaf-mottle virus by means of *Amphorophora rubi* Kalt.

Fig. 6. Shoot from plant of *Rubus occidentalis* var. Cumberland 6 weeks after transfer of *A. rubi* fed for 4 hr. on a source of leaf-mottle virus.

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SOME EXPERIMENTS AND FIELD OBSERVATIONS ON THE GERMINATION OF WILD OAT (*AVENA FATUA* AND *A. LUDOVICIANA*) SEEDS IN SOIL AND THE EMERGENCE OF SEEDLINGS

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(With Plate 24 and 4 Text-figures)

The distinguishing characters of *Avena fatua* and *A. ludoviciana* are described.

Pot experiments and field observations showed that most seeds of *A. fatua* germinated in spring and a few in autumn; hardly any germinated in summer or winter. Seeds of *A. ludoviciana* germinated in winter only. The greatest depth of sowing from which seedlings of either species reached the surface was 9 in., but seedlings from this depth were weak and yellow when they first appeared. *A. ludoviciana* gave more and sturdier seedlings than *A. fatua* from 6 and 9 in. There was no evidence of induced dormancy in seeds of *A. fatua* buried at depths down to 20 in. Germination of this species was hastened by monthly cultivation of the soil. The maximum survival of *A. fatua* was 3 years in pots and slightly longer in the field; seeds of *A. ludoviciana* in pots survived only 2 years. The two or three seeds of each spikelet of *A. ludoviciana* germinated in turn, starting with the largest, but the interval between germination of successive seeds varied.

Seedlings from freshly sown seeds of both species were more vigorous than seedlings from seeds which had been buried for a year or more. Germination and subsequent growth of both species took place in soil of pH 4·5 to 7·0 approx.

Introduction

Three species of wild oat are found as cornfield weeds in England at present; they are *Avena fatua*, *A. ludoviciana* and *A. strigosa*. The bristle-pointed oat, *A. strigosa*, is not important, as its seeds are non-dormant, and was omitted from these experiments.

A. fatua is the common wild oat and is widespread and abundant in the grain-growing areas of England. *A. ludoviciana* was first recorded in Britain about 15 years ago, since when it has become a serious pest in some parts, though it is often unrecognized owing to its general resemblance to *A. fatua*. The chief distinguishing character of *A. ludoviciana* is that all the seeds of a spikelet normally remain attached to one another when they ripen and fall from the plant, whereas in *A. fatua* all the seeds fall independently. In *A. ludoviciana* only the first and largest 'seed' of each spikelet has a round abscission-scar or callus at the base; the other 'seeds' of the spikelet end in stiff rachillae which must be broken to separate them from the first 'seed' and from each other. In *A. fatua* every 'seed' has a basal abscission-scar irrespective of its position in the spikelet. Spikelets of both species can contain

three 'seeds' but often only the first two develop. The tiny third 'seed' of *A. ludoviciana* is awnless, but the lemmas of all 'seeds' of *A. fatua* are awned (Pl. 24, fig. 1).

The husks of *A. fatua* vary in colour and hairiness. At least three different varieties are recognized: var. *pilosissima*, with brown, densely hairy lemmas; var. *pilosa*, with grey moderately hairy lemmas; and var. *glabrata*, with yellow lemmas glabrous except around the callus. The husks of *A. ludoviciana* are darker brown and more densely hairy than those of *A. fatua* var. *pilosissima*, shown in Pl. 24, fig. 1.

Both *A. fatua* and *A. ludoviciana* are annual weeds occurring chiefly in wheat and barley. They produce numerous seeds which ripen and are shed before the crop is harvested. Some of these seeds can remain dormant in the soil for over a year, germinating at intervals to renew the infestation. A knowledge of the behaviour of wild oat seeds in soil is therefore an essential basis for control measures. Some observations and experiments have been reported from France, Canada, U.S.A. and Germany, but none from Great Britain. *A. fatua* and *A. ludoviciana* both occur at Rothamsted, and the dormancy of the seeds of these two species is being investigated there by experiments and field observations. Those reported here deal with the retention of vitality and seasonal germination of seeds buried at different depths in soil and the ability of the resulting seedlings to develop into normal plants.

Pot experiments on *Avena fatua*

Method

Four pot experiments (A, B, C and D) were set up to investigate the survival and germination of seeds of *A. fatua* in soil under different conditions. All were sown with ripe seeds gathered by hand at Rothamsted in August 1944. 60 % of the seeds were var. *pilosa*, 27 % var. *pilosissima* and 13 % var. *glabrata*.

In Exp. A the seeds were mixed with wild-oat-free soil, which was then spread in a layer 2 in. deep in earthenware pans. At monthly intervals the soil in each pan was thoroughly mixed. In the other three experiments the seeds were buried in soil at depths from 1 to 20 in. and left undisturbed.

Exps. A, B and C were in a glasshouse where watering could be controlled but temperatures were higher than those prevailing in the field. Exp. D was out of doors, where high soil temperatures were avoided but heavy rain caused compacting of the soil and sometimes made it too wet. In the unusually cold spring of 1947 the soil in the pots of Exp. D was frozen solid for several weeks, but pots in the glasshouse never froze.

Exps. A and B were set up in autumn 1944. Numerous seedlings appeared even from the 5 in. sowing. A few seedlings reached the surface by way of the gap between the soil and the side of the pot. Exps. C and D with deeper sowings were therefore set up in spring 1945, keeping the seeds 1 in. away from the sides of the pot to ensure that the seedlings penetrated the whole depth of the soil above them.

The experimental details of the four experiments are summarized in Table 1. The pots of Exps. B and C were in three randomized blocks and Exp. D in two.

As the actual germination of seeds in soil could not be observed the results were based on the number of seedlings appearing above ground. Some seedlings may have died without reaching the surface, and this is most likely to have happened in the deeper sowings. Other seeds may have been destroyed by soil organisms or have been non-viable, although only apparently sound seeds were sown. It cannot therefore be assumed that all the seeds ungerminated on any date remained viable but dormant in the soil.

To see if any of the remaining seeds could be induced to germinate by being brought within 3 in. of the surface, pots of each depth of sowing in Exp. B were tipped out into shallow seed-boxes after 47 months. One block of Exp. C was treated similarly after 20 months and another after 44 months. The undisturbed pots and shallow boxes of soil from all experiments were kept for at least a year after the appearance of the last seedlings.

The difference between seeds germinating and seedlings appearing above the soil was probably least in Exp. A. In this experiment all seedlings were counted and removed at monthly intervals when the soil was being cultivated. Occasionally seedlings were dug up before they reached the surface, and these added to the total number of seedlings, as it was assumed that if they had been left undisturbed they would have come up eventually.

In Exps. B, C and D the seedlings were counted as they came up and then allowed to grow on until they became too numerous or until they were about to form seed. They were then cut off at ground level leaving the soil undisturbed. Occasionally they sprouted again, but were recognized by the broken tips of the leaves and cut off a second time.

Results

Experiment A

The total numbers of seeds appearing in five pans were 76, 72, 79, 79, 79, mean 77 %. Text-fig. 1 shows the percentage of seed which had given rise to seedlings by successive dates. The first seedlings came up 15 days after sowing. By the end of December 1944, i.e. after 2 months, 43 % of the seeds sown had given rise to seedlings. This was the biggest flush of germination occurring during the experiment and constituted more than half of all the seedlings produced. A further 17 % of the seeds produced seedlings during February and March 1945, and from then on throughout the summer a few seedlings appeared each month, with a slight increase in numbers in September, October and November. The few seedlings which came up in 1946 showed similar spring and autumn peaks of germination. The last two seedlings appeared in March and April 1947, $2\frac{1}{2}$ years after sowing.

After being kept for a further 18 months, the soil from the pans was washed through a fine sieve and the remaining coarse material was examined. A few husks were found but no intact seeds. It was concluded that the 23 % of seeds which were not accounted for had died without producing seedlings.

TABLE 1. *Experimental details of pot experiments on the effect of depth of sowing and cultivation on the germination of seeds of* Avena fatua *and* A. ludoviciana

Species and exp.	Type of pot	Size of pot (in.) Diam.	Size of pot (in.) Depth	Type of soil (free from wild oat seeds)	Site of exp.	No. of seeds per pot	No. of pots per treatment	Date of sowing	Exp. ended	Depth of sowing (in.)	Cultivation
Avena fatua A	Unglazed earthenware pan	7½	2	Poor clay from field	Glasshouse	100	5	31. x. 44	1. x. 48	Mixed with soil in layer 2 in. deep	Monthly
B	Mitscherlich (enamelled iron)	7½	7	Poor clay from field	Glasshouse	100	3	4. xi. 44	29. ix. 50	1, 2½, 5	Undisturbed
C	Glazed earthenware drain-pipe with bottom closed with wire netting	6	24	Fertile clayey loam from allotment	Glasshouse	75	3	22. ii. 45	29. ix. 50	3, 6, 9, 12, 15, 20	Undisturbed
D	Glazed earthenware drain-pipe standing on clay subsoil	9	22	Fertile clayey loam from allotment	Out-of-doors	100	2	27. ii. 45	29. ix. 50	3, 6, 9, 12, 15, 20	Undisturbed
Avena ludoviciana	As for Exp. C	6	24	Top-spit loam pH 4·5	Glasshouse	80 (=40 spikelets)	1	1. iv. 47	14. iii. 51	3, 6, 9, 12, 15, 20	Undisturbed

Experiments B and C

Emergence of first seedlings. Deep sowing delayed the appearance of the first seedlings by approximately 1 day for each inch of overlying soil (Table 3). This may have been due to the time taken for the seedlings to penetrate the soil and not to delay in germination. The slow start of the 5 in. sowing of Exp. B was due to inadequate watering, numerous seedlings appearing after the pots were soaked at the end of the first month. Seedlings appeared sooner after sowing in spring-sown Exp. C than in autumn-sown Exps. A and B, either on account of better growing conditions or because the seeds had after-ripened in storage.

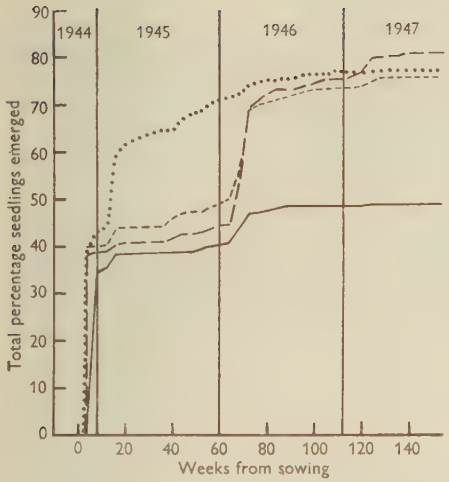
Length of coleoptile. The coleoptiles of seedlings from 3 in. projected ½–1 in. above the soil, and those of the 6 in. sowing just reached the surface; from 9 in. the coleoptiles never appeared, and the unprotected tips of the first leaves broke through the soil. Kirk & Pavlychenko (1932) found that seedlings of *A. fatua* reached the surface of the soil from depths down to 7 in., but that the length of the mesocotyl varied with the depth of sowing and the coleoptile node was always within 1 in. of the surface. Presumably, therefore, the maximum length of the mesocotyl is about 6 in., and if the seeds are sown deeper it cannot elongate sufficiently to allow the tip of the coleoptile to reach the surface of the soil.

Effect of depth of sowing on colour and sturdiness of seedlings. Seedlings from the 1 in. sowing always came up sturdy and green. The first flush of seedlings from 2½ and 3 in. were equally healthy but later plants seemed a little weaker, and by the second spring seedlings from 2½ in. were clearly weaker and paler green than those from 1 in. Seedlings from the 5 and 6 in. sowings were all yellowish green and appeared etiolated when they first came up, but most of them recovered by the two-leaf stage. Later batches showed more severe symptoms, and the last to appear were yellow and so weak that some did not come up vertically. Thus the seeds which were still viable after 1½ years had begun to lose their vitality.

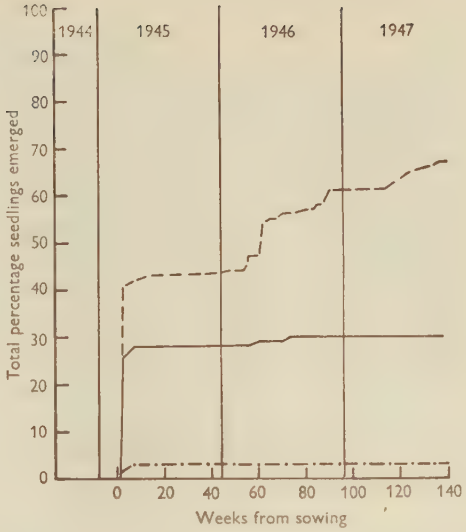
The only seedlings from 9 in. came up within 7 weeks of sowing. They were yellow and very weak, leaning against the side of the pot for the first few days (Pl. 24, fig. 3), but after a month they had recovered and appeared healthy. Seedlings continued to appear from 6 in. for 73 weeks, from 5 in. for 124 weeks and from 3 in. for 137 weeks, confirming that after prolonged burying of the seeds the resulting seedlings were less vigorous, since they could only penetrate thinner layers of soil.

Seasonal emergence of seedlings. The emergence of seedlings from all depths showed peaks in spring and autumn. The first flush of seeedlings was always the largest, and the numbers diminished fairly regularly with time (Text-figs. 1, 2). No seedlings appeared in the autumn of 1949 in the 5 in. sowing, probably because very few viable seeds remained. The 3 in. sowing gave no seedlings in the spring of 1947, 2 years after sowing, although viable seeds were still present in the soil.

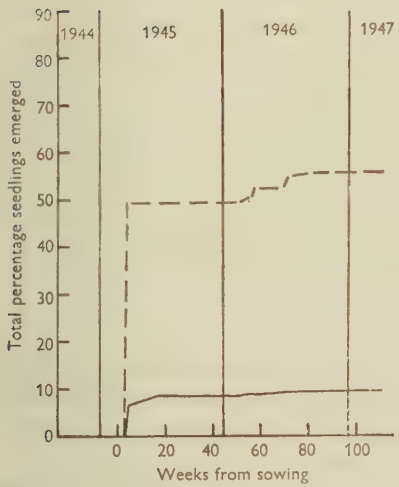
Effect of cultivation on emergence of seedlings. In Exp. B more seedlings appeared from all depths in the second spring than in the first (Text-fig. 1). Comparing this



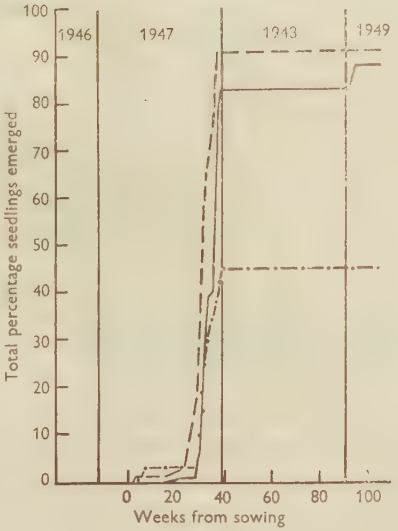
Text-fig. 1.



Text-fig. 2.



Text-fig. 3.



Text-fig. 4.

Text-figs. 1–4. Emergence of wild oat seedlings from seeds sown at different depths in soil.

Text-fig. 1. *Avena fatua*. Exps. A and B. Exp. A mean of five pans. Exp. B each depth mean of three pots.

Text-fig. 2. *Avena fatua*. Exp. C. Each depth mean of three pots.

Text-fig. 3. *Avena fatua*. Exp. D. Each depth mean of two pots.

Text-fig. 4. *Avena ludoviciana*. One pot for each depth.

· · · · · · · 0–2 in., cultivated monthly.
– – – – – – 1 in., undisturbed.
— — — 2½ and 3 in., undisturbed.
——————— 5 and 6 in., undisturbed.
—·—·—·— 9 in., undisturbed.

No more seedlings appeared after the periods shown, except for one seedling of *A. ludoviciana* from 3 in. in February 1951. The end of each curve therefore shows the final percentage seedlings emerged in that treatment.

germination in undisturbed soil with the emergence of seedlings in Exp. A, monthly cultivation appears to have hastened the breaking of dormancy of about 20 % of the seeds by 6–12 months. Quicker germination in cultivated soil was expected, as Bibbey (1935) found that autumn digging of compacted soil containing *A. fatua* increased germination in the following 15 days by 500 %.

Effect of date of sowing on emergence of seedlings. By April 1945 emergence in the 2½ in. depth of autumn-sown Exp. B was 41 %, 39 % occurring in the first month and a further 2 % in early spring. Exp. C was sown in late February 1945, and 41 % of the seeds sown at 3 in. gave seedlings in the first 3 weeks. Evidently

TABLE 2. *Total number of seedlings of* Avena fatua *which had appeared from different depths in Exp. B by the end of* 1945 *and by the end of* 1947

	29 December 1945				31 December 1947			
Block	1 in.	2½ in.	5 in.	Significant difference	1 in.	2½ in.	5 in.	Significant difference
1	52	46	47	—	74	86	58	—
2	51	48	25	—	77	79	30	—
3	45	40	49	—	76	77	59	—
Total	148	134	121	—	227	242	147	—
Mean	49·3	44·7	40·3	10·7	75·7	80·7	49·0	·11·5

100 seeds per pot, therefore means are also percentages.

Table 3. *Effect of depth of sowing on date of appearance of first and last seedlings of* Avena fatua

	Exp. B			Exp. C			Exp. D		
Depth of sowing (in.)	1	2½	5	3	6	9	3	6	9
Days to appearance of first seedlings	14	17	24	10	12	15	23	27	—
Weeks to appearance of last seedlings	128	140	124	137	73	7	83	72	—

those seeds which were ready to germinate in November had remained so in dry storage and had germinated in addition to those becoming germinable in spring.

The spring peaks of emergence in 1946 and 1947 were smaller and the autumn peaks larger in the 3 in. depth of Exp. C than in the 2½ in. depth of Exp. B (Text-figs. 1, 2). The last seedlings from 3 in. in Exp. C also appeared 3 months later than those of the 2½ in. depth of Exp. B. As Exp. C was sown 4 months later than Exp. B with seed from the same harvest, this suggests that the length of time spent in the soil is a factor in breaking dormancy.

Effect of depth of sowing on total number of seedlings produced. The numbers of seedlings appearing from the 1 and 2½ in. sowings of Exp. B did not differ significantly (Table 2). At both depths the final percentage of seeds which had given rise to seedlings closely resembled that in the frequently cultivated pans of Exp. A (Text-fig. 1). Fewer seedlings came up from 5 in., though in the first year the difference between 5 in. and the shallower sowings was not significant. During

February and March 1946 the 1 and 2½ in. depths gave four times as many seedlings as 5 in., and by the end of the experiment only 49 % of the seeds sown at 5 in. had produced seedlings, compared with 76 % from 1 in. and 81 % from 2½ in. This difference was highly significant (Table 2).

The 3 in. sowing of Exp. C gave only 66 % of seedlings. In view of the similarity of emergence from 2½ and 3 in. up to April 1945, it is unlikely that any seeds died in storage in the winter of 1944. Nor would the 15 % difference between 2½ and 3 in. have been due to increased depth, as the reduction between 3 and 6 in. was only 37 %. The difference was probably due to pre-emergence damage by insects or slugs, as in Exp. C twelve seedlings died after reaching the surface and some of them appeared to have been bitten. No insecticides were used, as their effect on the dormancy and viability of wild oat seeds was unknown.

Table 4. *Seedlings from seeds of* Avena fatua *sown at different depths in drain-pipes in glasshouse (Exp. C)*

	In the first 21 months after sowing					More than 21 months after sowing					
	In undisturbed pots					In undisturbed pots				In boxes	
Depth of sowing (in.)	Block 1	Block 2	Block 3	3 Blocks		Block 2		Block 3		Block 1	
	Total	Total	Total	Total	%	Total	%	Total	%	Total	%
3	43	51	36	130	59	0	0	13	17·3	10	13·3
6	7	32	27	66	29	0	0	0	0	0	0
9	0	1	5	6	3	0	0	0	0	1	1·3
12	0	0	0	0	0	0	0	0	0	1	1·3
15	0	0	0	0	0	0	0	0	0	4	5·3
20	0	0	0	0	0	0	0	0	0	2	2·7

Emergence from 6 in. was lower still, only 30 % of the seeds sown giving seedlings. This was 19 % lower than the 5 in. sowing, but again the difference may have been due to soil organisms. The difference between 3 and 6 in. was 37 %, compared with 32 % between 2½ and 5 in. in Exp. B, showing better agreement in the reduction due to depth than in the actual percentage of seedlings produced from comparable depths in the two experiments.

Only six seedlings came up from 9 in. and none came from greater depths. Thus in the conditions of Exp. C, 9 in. was the greatest depth of soil which even the most vigorous seedlings of *A. fatua* could penetrate (Table 4).

Length of time seeds can remain viable but dormant in soil. The last seedlings appeared in the undisturbed pots of both experiments 32 months after sowing. No seedlings came up when pots were tipped out into shallow boxes approximately 4 years from sowing, showing that all seeds which had not germinated within 4 years had died.

Block 1 of Exp. C was put into boxes 21 months after sowing. Table 4 gives the number of seedlings subsequently appearing in the boxes and undisturbed pots.

The last seedlings came up in the boxes 38 months after sowing and 6 months later than in undisturbed soil.

Approximately 15 % of the seeds sown at 3 in. gave seedlings after 21 months in undisturbed and in churned-up soil (Table 4). The 3 in. pot which gave no seedlings after 21 months had the highest germination in the previous period and probably contained no more viable seeds. The mean survival of seed buried deeper than 3 in. was only 2 %, as shown by the boxes. No seedlings appeared from these depths in undisturbed soil, probably on account of lowered vitality due to the age of the seed. The differences between the deeper sowings were too small to be important.

No evidence of induced dormancy. There was no evidence in these experiments of induced dormancy in seeds of *A. fatua* buried at depths down to 20 in. This is contrary to the opinion of Rabaté (1913) and Cates (1917). Slight evidence of induced dormancy was obtained at the end of the first year of Duvel's buried weed-seed experiment (Toole & Brown, 1946) when 10 % more seeds of *A. fatua* germinated from a pot buried at 42 in. than from pots buried at 8 or 22 in., but the difference is of no practical importance, as field soil is never cultivated so deeply. No seeds survived being buried at these depths for 3 years.

Survival of Avena fatua *compared with other weeds present in the soil.* By February 1949 the boxes from Exp. B contained seedlings of other weeds, particularly *Stellaria media*, while the adjacent undisturbed pots had none. Similar seedlings had been removed from all pots at the beginning of the experiment but no more had appeared. The seeds of *S. media* had therefore survived longer in undisturbed soil than those of *Avena fatua*. But Brenchley & Warington (1930) showed that *Stellaria media* survives only 2 years in frequently mixed soil. The deeply buried and undisturbed seeds of *S. media* therefore showed induced dormancy although *Avena fatua* in the same experiment did not show it.

The boxes of Exp. C produced many seedlings of *Lamium* sp. probably *L. purpureum*. *Ranunculus* sp., *Capsella bursa-pastoris* and Gramineae other than *Avena fatua* were fairly numerous, and *Rumex* sp., *Euphorbia* sp. and *Anagallis arvensis* were occasional. The undisturbed pots were weed-free and moss-covered. The weeds in the boxes must have grown from buried dormant seeds stimulated to germinate by the disturbance of the soil. Some of these species are known to have a long period of survival in cultivated soil (Brenchley & Warington, 1930).

Experiment D

This experiment gave slightly different results from Exp. C, probably due to outdoor conditions. The first seedlings appeared later (Table 3). None came up from 9 in. or from the deeper sowings. The coleoptiles did not reach the surface even from 3 in., but the leaves from this depth were green when they first came up. Seedlings from 6 in. came up yellow as in Exp. C but recovered and developed into sturdy plants with normal ears, though ear emergence was slightly delayed (Pl. 24, fig. 2 and Table 5).

10% more seedlings appeared in the first flush from 3 in. than from the same depth in the glasshouse, but no seedlings came up in the first autumn (Text-fig. 3). The spring and autumn peaks of the second year were smaller than in Exp. C. The last seedlings appeared on 3 October 1946, 83 weeks after sowing, bringing the percentage of seedlings to 56, against 67 % in 137 weeks in the glasshouse. The hard and prolonged frost of early spring 1947 may have killed any ungerminated seeds remaining in the soil.

The 6 in. sowing out-of-doors gave only 9 % of seedlings against 29 % in the glasshouse. Largely due to this, the difference in percentage of seedlings between the 3 and 6 in. sowings was greater out-of-doors. Germination from 6 in. continued for the same length of time as in the glasshouse, but there was no peak of seedlings emergence out-of-doors in the first autumn. The reason for this is unknown, but since the 3 in. sowing of Exp. D also gave no seedlings in the autumn of 1945 it may have been connected with the weather.

TABLE 5. *Plants of* Avena fatua *removed from* 3 *and* 6 *in. sowings of Exp. D on* 26 *June* 1945

(See also Pl. 24, fig. 2.)

Depth of sowing	Block no.	Total plants	Total culms	Total ears emerged	Maximum height (cm.) to top of tallest ear
3 in.	1	29	49	27	77
	2	31	53	41	78
6 in.	1	6	26	3	76
	2	10	20	9	66

POT EXPERIMENT ON *AVENA LUDOVICIANA*

Method

An experiment on the effect of depth of sowing of *A. ludoviciana* on the emergence of seedlings was set up on 1 April 1947 with one pot for each depth of sowing (Table 1). The depths were the same as in Exp. C on *A. fatua* and the pots stood in the same glasshouse. The seeds of *A. ludoviciana* were collected at Rothamsted in August 1946 and stored in the laboratory during the winter. The soil was screened top-spit loam of pH 4·5. No lime was added. The soil acidity did not harm *A. ludoviciana*, although it severely damaged tomatoes, cabbages, white mustard and various annual flowers sown in similar soil at about the same time.

Results

As in *A. fatua*, 9 in. was the greatest depth from which seedlings appeared. The percentages of seedlings from 3, 6 and 9 in. were much higher for *A. ludoviciana* than for *A. fatua*, the difference between the species being 42 % at 9 in. 58 % at 6 in. and 24 % at 3 in. This may have been due partly to greater viability of the *A. ludoviciana* seed, as the highest germination obtained from *A. fatua* was 81 % (Exp. B).

The insect damage suspected in Exp. C would also increase the difference in percentage of seedlings between it and this experiment, but even allowing for this the difference between species at 6 and 9 in. must be significant.

The final percentage of seedlings of *A. ludoviciana* from 3 and 6 in. were almost identical, and 45 % of the seeds sown at 9 in. gave seedlings (Text-fig. 4). The seedlings of *A. ludoviciana* therefore seem to possess considerably greater powers of penetrating overlying soil than do those of *A. fatua*. Since growth through the soil takes place before any photosynthesis is possible, the food reserve in the seed must be important. The seeds of *A. ludoviciana* are larger than those of *A. fatua* (Pl. 24, fig. 1 and Table 6), and it therefore seems reasonable that they should give seedlings with greater penetrating power. Perhaps the 45 % of seeds of *A. ludoviciana* which gave seedlings from the 9 in. sowing were all large first seeds. The smaller second seeds must have given rise to seedlings from 6 in., since 88 % of the seeds sown produced seedlings but only 50 % of them were the large first seeds.

TABLE 6. *1000-grain weight in February 1951 of air-dried seeds of* Avena fatua *and* A. ludoviciana *with husks removed*

A. fatua		*A. ludoviciana*			
	Dry weight (g.) of 1000 seeds		Dry weight (g.) of 1000 seeds		
Harvested	Mixed	Harvested	1st seeds	2nd seeds	3rd seeds
1944	12·93	1946	23·39	14·92	6·68
1950	11·87	1950	17·60	10·47	—
Mean	12·40	Mean	20·50	12·70	6·68

Seedlings of *A. ludoviciana* from the 9 in. sowing were yellow when they first appeared but turned green within a week of reaching the light. Seedlings from 6 in. were green from the first, but a little weaker than those from 3 in. As in *A. fatua*, the coleoptile appeared above ground from 3 in., just reached the surface from 6 in. and did not come through at all from 9 in.

Almost all seedlings of *A. ludoviciana* emerged in winter, in contrast to the spring and autumn peaks of *A. fatua*. Only three seedlings appeared within 7 weeks of sowing in spring and no more came up until September. Most seedlings appeared in October, November and December 1947, 7–9 months after sowing. This contrasts sharply with *A. fatua*, which gave a flush of seedlings within a month of sowing either in spring or autumn.

No more seedlings appeared from the 9 in. sowing throughout the remaining 3 years of the experiment, but four seedlings came up from 6 in. in January 1949, i.e. in the second winter, and one from 3 in. in February 1951, i.e. in the fourth winter. In this experiment, therefore, almost all of the seeds of *A. ludoviciana* remained dormant in the soil for a shorter time than those of *A. fatua* in Exps. A–D, most germinating within 9 months of sowing and all except one within 2 years.

Unfortunately, the seeds were in dry storage for the first winter after harvest, so it is not known how many would have germinated then if given suitable conditions.

The 1949 seedlings from 6 in. were yellowish when they emerged and all except one died, though the survivor grew well and when removed on 17 May was nearly in ear. Prolonged burying of the seeds evidently reduced the vigour of the resulting seedlings as in *A. fatua*.

Comparison of results of pot experiments with field observations

Observations have been made on the emergence of wild oat seedlings under field conditions every year since 1945, for comparison with the results of the pot experiments. Most of the observations at Rothamsted were made on the two classical cereal experiments on Broadbalk and Hoosfield; in order to avoid damage to the crop the observations were made from outside the plots, no counts were attempted and seedlings were only dug up from the paths. The most complete records were made in 1945–6 and were confirmed in subsequent years.

Winter wheat has been grown continuously on Broadbalk field with different manurial treatments since 1843. In recent years the field has been divided into five sections, one of which is fallowed each year to control weeds. This field thus provides a good opportunity for studying the effect of 1 year's fallow on the build-up of cornfield weeds, including wild oats.

Spring barley has been grown on Hoosfield yearly since 1856, on plots receiving different combinations of manures. No regular fallowing system has been introduced, but the whole field was fallowed in 1943 in an attempt to control wild oats.

Similar experiments on the continuous growing of winter wheat and spring barley have been carried on at Woburn. The site of the barley experiment was fallowed from August 1946 to March 1950 in an attempt to eradicate a heavy infestation of *A. fatua*. Dr H. H. Mann obtained figures for the survival of seeds of *A. fatua* in this frequently cultivated field soil. His kindness in making these observations and allowing them to be published here is gratefully acknowledged.

Periodicity of germination

Germination of *A. ludoviciana* on Rothamsted farm took place almost entirely in winter, between mid-October and the end of January, thus confirming the result of the pot experiments. For example, on Broadbalk on 28 September 1945, no wild oat seedlings were visible, and when wheat was sown on 19 and 20 October the cultivations destroyed any seedlings which were present, but by 30 October the most severely infested plot was green when seen from a distance, due to numerous seedlings of *A. ludoviciana*. The other plots had fewer seedlings. On 3 December 1945 the most severely infested plot was so covered with seedlings of *A. ludoviciana* that the rows of wheat could not be seen and the path could scarcely be distinguished from the sown plot. On 5 February 1946 occasional young seedlings were found

among the older plants, which by now had at least three leaves and were beginning to tiller. No young seedlings were found on 27 March, and after this both crop and wild oat plants grew rapidly, preventing further observations on emerging seedlings. According to Chevalier (1925), in south-west France this species germinates between December and April, thus having a single yearly peak as at Rothamsted but about 2 months later.

Relatively few seeds of *A. fatua* germinated in autumn on Rothamsted farm, and the resulting plants seemed to make little growth during the winter. Most germination took place in March and April, either before or after the sowing of spring crops. For example, on Hoosfield on 28 September 1945, a few seedlings were found at the one- or two-leaf stage, and by 3 December 1945 seedlings were still infrequent.

TABLE 7. *Seedlings of* Avena fatua *appearing on an infested field at Woburn, bare fallowed after barley harvested in* 1946 *and cultivated at intervals*

Period	Seedlings per acre	Greatest depth from which seedlings reached surface of soil
Aug. 1946 to Aug. 1947	No records	—
12 Aug. 1947	A few	—
30 Oct. 1947 to 22 Feb. 1948	44×10^5	At least 7 in.
22 Feb. 1948 to 14 Apr. 1948	36×10^5	$6\frac{1}{2}$ in.
14 Apr. 1948 to 7 May 1948	17×10^5	—
7 May 1948 to early July 1948	No record	—
Early July 1948 to 23 Aug. 1948	Hardly any	3 in.
23 Aug. 1948 to 22 Sept. 1948	121	4 in.
22 Sept. 1948 to 20 Nov. 1948	584	—
20 Nov. 1948 to 3 Mar. 1949	33×10^3	—
3 Mar. 1949 to 31 May 1949	15×10^3	—
31 May 1949 to July 1949	No record	—
July 1949 to 7 Nov. 1949	4×10^3	$2\frac{1}{2}$ in.
7 Nov. 1949 to 12 Mar. 1950	375	—

Counts made just before each cultivation. Plants surviving previous cultivation not included in counts.

A. fatua vars. *pilosa* and *pilosissima* with approx. 1 % var. *glabrata*.

No *A. ludoviciana* present.

Most had only one leaf, and those dug up came from deeply buried seeds. On 5 February 1946 the seedlings of *A. fatua* were no more numerous and had only one leaf. Barley was sown in the last week in March, leaving the field clean. During April a flush of *A. fatua* seedlings came up among the germinating barley, and by 1 May all the wild oat seedlings found had at least two tillers. As the barley was also tillering and plants were 3–4 in. high, even the larger wild oat plants were only seen on close examination, and it was possible for very young seedlings to be overlooked.

Dr H. H. Mann's observations on the emergence of *A. fatua* at Woburn (Table 7) showed that as at Rothamsted and in the pot experiments, the chief period of germination of this species was in spring, with a second and considerably

smaller peak in autumn. A similar yearly pattern of germination was shown in Saskatchewan (Bibbey, 1935; Chepil, 1946), though the spring peak was slightly later than at Rothamsted, as might be expected from the severity and duration of the Canadian winter. Most seedlings appeared between mid-April and mid-June, i.e. after the spring cereals had been sown.

Effect of date of sowing of crop on species of wild oat present

At Rothamsted, *A. ludoviciana* was almost entirely confined to autumn-sown crops, chiefly winter wheat. *A. fatua* occurred in both autumn- and spring-sown crops, but was more plentiful in the latter. For example, the wild oats on Broadbalk are mainly *A. ludoviciana* with a few areas of *A. fatua*, while Hoosfield has only *A. fatua*.

Another field became infested with both species of wild oats during a series of experiments on winter wheat. In 1949 it was divided into four strips, three being sown with wheat, barley and oats on 1 and 2 November 1949, and one with ryegrass on 15 March 1950. By 22 June 1950 wild oat plants on all four strips were developing ears. Those in the cereals were almost entirely *A. ludoviciana* and those in the ryegrass were *A. fatua*, with very occasional plants of *A. ludoviciana*. The different sowing dates had thus had a selective effect on the species of wild oats reaching maturity on the four strips, the spring sowing having killed the plants of *A. ludoviciana* already present and no more having germinated after that date. Presumably most of the seedlings of *A. fatua* appearing in spring among the winter cereals and *A. ludoviciana* had been killed by competition.

At Woburn, the wheat and barley experiments corresponding to Broadbalk and Hoosfield were side by side on the same field. The spring-sown barley was infested with *A. fatua*, but the adjacent autumn-sown wheat was almost free of wild oats. Presumably this was due to the different size of the crop plants at the time of germination of the wild oats. Fewer seedlings of *A. fatua* had established themselves in the winter wheat than in the corresponding experiment at Rothamsted. *A. ludoviciana* does not occur at Woburn.

Chevalier (1925) stated that autumn rye, *Trifolium incarnatum*, and maize seemed to inhibit the germination of *Avena fatua*, but that the wild oats reappeared when wheat was sown again. The sowing dates of maize (May) and *Trifolium incarnatum* (August) are in the summer period when *Avena fatua* does not normally germinate. Their antagonism to *A. fatua* may therefore be explained in the same way as the influence of the crop on the species of wild oat present on fields at Rothamsted and Woburn. But this does not account for the antagonism of autumn rye. Osvald (1950) believes that there is a root exudate from rye which inhibits germination of *A. fatua*. On the other hand, severe competition from the crop can restrict the development of plants of *A. fatua* (Pavlychenko & Harrington, 1934) as autumn rye ripens early, possibly before the wild oat ears are visible above the crop. Thus the absence of wild oats in autumn rye might not be due to the suppression of germination.

SURVIVAL OF DORMANT SEEDS IN THE FIELD

A severe infestation of *A. ludoviciana* has developed on Broadbalk since the introduction of the fallowing system, and in some areas *A. fatua* is established. One year's intensive cultivation is therefore insufficient to eradicate dormant seeds of either species from the soil. This was confirmed for *A. fatua* on Hoosfield, which was bare fallowed in 1943 without effectively reducing the wild oats in the 1944 barley crop.

The emergence of *A. fatua* seedlings from buried seeds in frequently cultivated soil was observed at Woburn by Dr H. H. Mann. After barley was harvested in 1946 a field infested with *A. fatua* was fallowed until 1950, with frequent cultivations to destroy successive crops of wild oat seedlings. Seedlings were counted just before most of the cultivations, and on five occasions seedlings were dug up to see how deeply buried were the seeds from which they sprang (Table 7). No fresh seeds reached the soil after the harvest of 1946, so the seedlings germinating in spring 1950 came from seeds which had been in the ground for at least $3\frac{1}{2}$ years. This is a year longer than the seeds survived in the pans of Exp. A kept in a glasshouse and cultivated every month, or in the undisturbed pots of Exp. B and slightly longer than the maximum survival in Exp. C. This was probably due to the field counts being based on a very much greater number of seeds than the pot experiments. Nearly 10 million seedlings per acre were recorded between 30 October 1947 and 7 May 1948, over a year after the last seeds reached the soil, so the 375 seedlings per acre between 7 November 1949 and 12 March 1950 represent a very small fraction of the seeds originally present. It is therefore unlikely that a pot experiment with only 500–1000 seeds would give any seedlings $3\frac{1}{2}$ years after sowing, even if survival were equal to that in the field. Similar results were obtained by Chepil (1946) with *A. fatua* in Saskatchewan, germination of seeds sown in soil out-of-doors falling from 80 % in the first year to about 0·2 % in the fourth year after sowing.

The only information we have on the survival of *A. fatua* seeds in undisturbed field soil came from a farmer at Furneux Pelham, Herts. A 4-year ley was ploughed up in 1949 and winter wheat was sown. A sprinkling of *A. fatua* ears appeared in 1950. No dung or compost in which wild oat seeds could have been introduced had been used, and wheat from the same seed sown on an adjoining field was free of wild oats. There had been a few wild oat plants when the field was last in corn. The seeds of *A. fatua* seemed therefore to have survived in the soil for 4 years under ley. These results on the survival of *A. fatua* under different conditions in the field agree with observations in France, Canada and U.S.A. (Rabaté, 1913; Clark, 1914; Cates, 1917).

The maximum length of time that seeds of *A. ludoviciana* can survive in field soil is not yet known, but the relative duration of dormancy in the three seeds of the spikelet was observed on Broadbalk by digging up seedlings from the paths on

sections after crop and after fallow and examining the seeds from which they grew. The first seed to germinate was normally the largest or outermost. This was followed by the second seed, and finally by the tiny third seed if this was present. As the oldest seedling in Pl. 24, fig. 4, had only three leaves, the difference in date of germination between first and third seeds was probably not more than 2 or 3 months, but germination of the second seed did not always follow so soon after the first. Table 8 and Pl. 24, fig. 5, show that a year might elapse between the germination of the two seeds of a spikelet.

In germination tests Coffman & Stanton (1938) found that the large primary seed of each spikelet of *A. ludoviciana* germinated more readily than the smaller secondary seed. The two seeds of *A. fatua* differed in the same way (Johnson, 1935; Chepil, 1946). As the primary and secondary seeds of *A. fatua* separate when ripe and differ only in size, it was not possible to follow their germination in the field.

TABLE 8. *Germination of first and second seeds of* Avena ludoviciana *on Broadbalk, 3 December* 1945

Condition of spikelets		No. of spikelets	
First seed	Second seed	After crop	After fallow
Germinated	Germinated but seedling younger than from 1st seed	3	0
Germinated	Present, healthy but dormant	9	1
Germinated	Present, but probably not viable	1	0
Present, but probably not viable	Germinated	1	0
Absent	Germinated	0	7
	Total spikelets examined	14	8

Random sample of seedlings from farmyard manure plot, dug up to show spikelets from which they grew.

Only intact seedlings included. No three-seeded spikelets found.

After crop most spikelets have been in the ground 4 months and after fallow at least 16 months.

DEPTH FROM WHICH SEEDLINGS REACH THE SURFACE

Investigation was difficult in the heavy clay soil of Rothamsted. Seedlings of *A. ludoviciana* were found arising from seeds on the surface or buried at depths down to 6 in. Seedlings from greater depths usually broke, but two were obtained intact from 7 in. and one from 8 in. Seedlings of *A. fatua* were dug up intact down to 5 in., and broken seedlings may have come from greater depths.

Chepil (1946) said that seeds of *A. fatua* would not germinate when lying on the surface of the soil, but Bibbey (1948) found that in the autumn dormant seeds of *A. fatua* after-ripened more quickly on the surface than when buried at 1–3 in. No seeds were left on the surface in the Rothamsted pot experiments. In the field, seedlings of *A. fatua* generally arose from seeds covered, however lightly, with soil. The large spikelets of *A. ludoviciana* were occasionally found on the surface with one or more seeds germinated.

Occasionally yellowish seedlings with only one leaf were found. Some came from deeply buried seeds, but others were due to insect damage or waterlogged soil. Healthy seedlings with several leaves were often traced to 6 in. or deeper. Seedlings from deeply buried seeds in the field were probably weak and yellowish when they emerged, but developed into healthy green plants as in the 5 to 9 in. sowings in pots.

Dr Mann's Woburn observations (Table 7) showed that seeds of *A. fatua* which had been buried for 1½ years gave seedlings which could penetrate a maximum depth of 7 in. of the sandy soil, but after 3 years maximum penetration was only 2½ in. This is comparable to Exp. C, where the last seedlings from 3 in. came up 2½ years after sowing and from 6 in. 2 years after sowing, but the only seedlings from 9 in. emerged within 7 weeks of sowing. Cultivation of the field soil every 2–4 months must have altered the depth at which most seeds were buried and cancelled out any effects of depth of burying on dormancy, even if there were such an effect, though the pot experiments indicated that depth of burying down to 20 in. did not affect dormancy.

The loss of vigour of wild oat seedlings with increasing age of the seed has not previously been recorded. Barton & Garman (1946) investigated the effect of long storage of the seeds on the yield of five cultivated plants, and concluded that the conditions of storage rather than the age of the seed affected the vigour of the resulting plants.

Tolerance of soil acidity

At Rothamsted seedlings of *A. ludoviciana* were numerous in soil of pH 6·0 to 7·0 approx., showing by comparison with the pot experiments that acidity was not essential to the germination of *A. ludoviciana* and that a considerable range of pH was tolerated.

At Rothamsted and at Woburn *A. fatua* grew on plots which were too acid to support barley, as well as on soil of the more usual pH range of 6·0 to 7·0 approx. *A. fatua* germinated and grew freely at Rothamsted on a small area at pH 4·5. At Woburn plants were few and small on a plot with pH 4·4, larger and rather more numerous on an adjacent plot with pH 5·1, and very plentiful at pH 5·8 and 5·9. This suggests that *A. fatua* germinated less well in acid soil at Woburn than at Rothamsted, but the difference may have been due to competition with the dense mat of *Agrostis* sp. covering the acid plots at Woburn. The acid plot at Rothamsted was almost bare except for the wild oats.

Discussion

The results of the pot experiments and field observations described here agree with those of previous workers in showing a single winter peak of germination for *A. ludoviciana* and two peaks for *A. fatua*, the larger one in spring and the other in autumn, and in indicating that the maximum survival of *A. fatua* in soil is about

4 years. Cates (1917) put the maximum survival of *A. fatua* under favourable conditions at 4–6 years. Duvel's experiment (Toole & Brown, 1946) gave a survival of less than 3 years, but only 100 seeds were tested for each depth, probably too few to demonstrate the survival of a small percentage. The Rothamsted experiments confirmed that cultivation stimulated germination of *A. fatua* (Bibbey, 1935) and showed that in the field as well as in the incubator (Coffman & Stanton, 1938) the primary seed of *A. ludoviciana* germinated more readily than the secondary.

The Rothamsted results on the emergence of seedlings of *A. fatua* from seeds buried at depths down to 7 in. agree with the observations of Kirk & Pavlychenko (1932). Cates (1917) said that seedlings of *A. fatua* would come up from 3 to 4 in., but that deeper-sown seeds might remain dormant until brought nearer to the surface. Rabaté (1913) also thought that deep ploughing caused buried seeds to remain dormant, and in Duvel's experiment (Toole & Brown, 1946) at the end of the first year 10% more seeds had survived at 42 in. than at 8 or 22 in. The Rothamsted results showed no induced dormancy at depths down to 20 in., the deepest sowing tested. They thus agree with Duvel's figures as far as they overlap, and it is doubtful whether a 10 % difference between two batches of 100 seeds is significant. Cates did not support his statement with experimental evidence, and the abstract of Rabaté's paper does not mention experiments; it is therefore impossible to say whether they were dealing with conditions very different from those at Rothamsted, or whether their statements were based on insufficient evidence.

There is no previous work on the effect of depth of sowing on the emergence of seedlings of *A. ludoviciana*, nor on the length of survival of its seeds in soil. The Rothamsted results indicate that compared with *A. fatua* a higher percentage of its seedlings come up from depths of 6–9 in., and that the maximum depth from which seedlings reach the surface is less than 12 in. The seeds survive more than 1 year in soil, but the maximum survival may be shorter than in *A. fatua*, possibly only 2 years.

The effect of the length of time the seeds have remained dormant in soil on the vigour of the resulting seedlings has not been recorded previously for either *A. fatua* or *A. ludoviciana*, nor has the tolerance of seeds and plants of both species to a wide range of soil acidity.

The experiments and observations described above dealt only with what happened to wild oat seeds in soil and not with how or why it happened. The results showed good agreement with those recorded in Canada, U.S.A. and France, so it seems legitimate to apply discoveries of the cause of dormancy made on the wild oat strains of these countries in interpreting the behaviour of the Rothamsted and Woburn wild oats.

Dormancy in *A. fatua* develops after fertilization of the ovule and depends on the genetic constitution of the embryo (Johnson, 1935). It is due to the impermeability of the seed coat to oxygen (Atwood, 1914), but the response of dormant seeds to oxygen may depend on the stage of after-ripening they have reached (Bibbey, 1948).

The degree of dormancy is also affected by the position of the seed in the panicle, the first seeds to ripen being the quicker to germinate (Johnson, 1935). Johnson (1935) also found that among primary seeds of *A. fatua* the heavier grains germinated more readily than the lighter, though he suggested that the heavier seeds might have been hybrids with the non-dormant *A. sativa*. Manganese deficiency of the parent plants can result in the formation of smaller and more readily germinated seeds of *A. fatua* and probably of *A. ludoviciana* (Thurston, 1951).

In view of the numerous factors affecting the development of dormancy in *A. fatua*, it is hardly surprising that batches of seed collected at apparently the same stage of maturity in two successive years on the same field can differ by 90 % in the proportion of dormant seeds present at harvest (Bibbey, 1948). The results obtained from experiments on one batch of seeds may not apply to other batches of different dormancy, but the results of the Rothamsted pot experiments agreed reasonably well with field observations and published data and appear to be of fairly general application.

Besides these internal and developmental factors, dormancy in *A. fatua* is also affected by external conditions, such as the length of time since the seeds were harvested (Johnson, 1935; Coffman & Stanton, 1938), conditions during that time (Bibbey, 1948; Johnson, 1935) and probably the temperature at which germination is tested. Soaking in dilute potassium nitrate hastened germination, but the effect of light was inconclusive (Johnson, 1935).

The dormancy determined in any sample of seeds of *A. fatua* is thus the result of many factors operating from the fertilization of the ovule to the germination of the after-ripened seed. It is therefore impossible to say which were responsible for the results under uncontrolled conditions such as those of the Rothamsted experiments, though suggestions can be made to be tested in subsequent experiments. Oxygen supply may have been important in the depth-of-sowing pot experiments, especially as the soil became compacted, though air could enter at the bottom of the pots in all except Exps. A and D, and the deepest sown seeds were therefore not the farthest away from the air. Improved soil aeration in the cultivated pans may have stimulated germination, and damage to the seed coat during mixing may also have helped. In the undisturbed pots some other agency must have ruptured the coats of dormant seeds. Rotting by soil micro-organisms seems probable, by analogy with *Symphoricarpos*, in which dormancy is overcome by fungi attacking the seed coat (Flemion, 1934; Pfeiffer, 1934). This is supported by the fact that the spring and autumn peaks of germination coincide roughly with the peaks of activity of bacteria and protozoa in field soil at Rothamsted (Cutler, Crump & Sandon, 1922).

The difference between *A. ludoviciana* and *A. fatua* in periodicity of germination indicates that the two species cannot be identical in their requirements for breaking dormancy. There is no information in the literature about the causes of dormancy in *A. ludoviciana*, but experiments on this species are in progress.

Fig. 1.

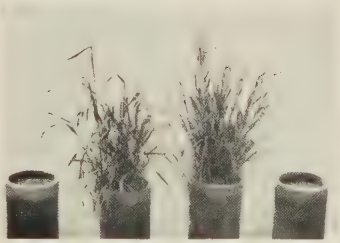


Fig. 2.

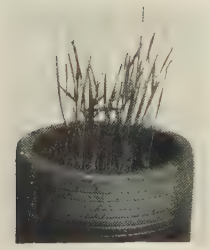


Fig. 3*a*.

Fig. 3*b*.

Fig. 3*c*.

Fig. 4.

Fig. 5.

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EXPLANATION OF PLATE 24

Fig. 1. Wild oat seeds. Top row *Avena fatua*. Bottom row *A. ludoviciana*. Left to right whole spikelet, first 'seed', second 'seed', third 'seed' (in *A. ludoviciana* only), showing the abscission scar at the base of both 'seeds' in *A. fatua* but only the first 'seed' of *A. ludoviciana*.

Fig. 2. Plants of *A. fatua* from seeds sown 6 and 3 in. deep on 27 February 1945, photographed 26 June 1945. Plants from 6 in. are sturdy but slightly later in coming into ear than those from 3 in. No seedlings came up in the 20 in. sowing (on left) or the 12 in. sowing (on right).

Fig. 3 *a*, *b*, *c*. Seedlings of *A. fatua* from seeds sown 3, 6 and 9 in. deep (Exp. C) on 22 February 1945. Photographed 15 March 1945. Seedlings from 6 in. slightly weak. Seedlings from 9 in. few, pale and very weak.

Fig. 4. Spikelet of *A. ludoviciana* from Broadbalk, 5 February 1945. All three seeds have germinated. The oldest seedling is from the largest seed and the youngest from the smallest.

Fig. 5. Seedlings of *A. ludoviciana* from Broadbalk, 5 February 1945. Two on the left after crop. Spikelets have been in the soil about 7 months. The first seed has germinated and the small second seed is still attached but dormant. Two on the right after fallow. Spikelets have been in the soil at least 18 months. First seeds germinated in the first season and the seedlings were destroyed by cultivation. Second seeds have germinated more recently. The broken rachilla at the base of the seed on the extreme right shows that it is not a first seed.

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OBSERVATIONS ON BROWN ROT (*SCLEROTINIA FRUCTIGENA*) OF APPLES IN RELATION TO INJURY CAUSED BY EARWIGS (*FORFICULA AURICULARIA*)

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(With Plate 25 and 6 Text-figures)

Observations in West Midlands orchards in 1947–50 on the apple varieties Cox's Orange Pippin and Laxton's Superb showed that brown rot was often associated with shallow holes in the fruit. Laboratory experiments with undamaged apples proved that earwigs (*Forficula auricularia*) could cause this type of injury.

Bands of sacking soaked in BHC placed around trunks of Laxton's Superb trees in 1949 and 1950 trapped many earwigs; less brown rot occurred in the banded than on control trees. A survey of picked fruit in 1950 showed close correlation between degree of earwig damage and amount of brown rot.

Earwig damage was appreciable in fruit from ten out of thirteen grass orchards, but was negligible in fruit from nine arable plantations.

Following complaints from a number of growers of heavy losses due to brown rot (*Sclerotinia fructigena*) of apples, particularly in the varieties Laxton's Superb and Cox's Orange Pippin, a preliminary survey of orchards was made in 1947. This revealed the difficulty of making precise estimates of losses due to brown rot and showed that the problem varied in intensity from orchard to orchard even with the same varieties. Wormald & Moore (1945) showed that brown rot followed injuries to the fruit caused by a number of agents, one of the most important being codling moth (*Cydia pomonella* L.). An attempt was therefore made to obtain some information on the precursors of the disease in several orchards in Worcestershire where losses were heavy. Identifying the initial cause of injury in an apple already substantially rotted proved to be difficult. Codling moth, however, appeared to have been well controlled in the orchards examined, but a number of fruits showed small, circular, shallow holes of unknown origin. Attempts were therefore made to obtain more precise information on the losses caused by brown rot and the factors influencing its incidence.

Sites and methods

Laxton's Superb apples in four orchards near Evesham, A, B1, B2 and C, were selected for recording. The tree forms, ages and managements were as follows:

A: bush, 15 years, first grassed in 1948.

B1: bush, 30 years, in grass.

B2: half-standard, 30 years, in grass.

C: bush, 12 years, arable with some grass around trunks.

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An adequate programme of sulphur sprays was applied in all the orchards.

The method used for recording brown rot in 1948 and 1949 was a modification of one devised by M. H. Moore for the Disease Measurement Committee of the British Mycological Society. In each orchard six trees were selected at random, and a main branch was marked on each of the north, east, south and west sides of the tree. The number of apples present, including those on secondary branches, was counted in mid-July. The total length of branch was measured as accurately as possible. Visits were made to the orchards at about 10-14-day intervals, and any apples infected with brown rot were removed from the marked branches. Apples with secondary brown rot arising through contact with other infected apples were classified separately. The amount of primary and secondary brown rot was calculated as a percentage of the number of apples present at the beginning of the observations.

In 1950, a different method of recording brown rot was adopted, as accurate counting of the number of apples on a complete branch had proved very difficult, and also there was great variation in the number of apples under observation on each tree. Eight branches which could be observed from the ground were chosen on each tree. Starting from the tip of the branch and including any laterals joining it, twenty-five apples were counted. At this point an aluminium label was tied on and the branch was marked with white paint. The branches were examined at intervals of 8 days or less.

As will be shown below, the relation between brown-rot infection and earwig infestation became a question of major importance. To restrict the access of earwigs to the trees the method used was trunk banding with treated sacking. Treatment of the sacking by steeping in DDT paste (made with 25 % wettable powder diluted with water to give 5 % actual DDT) proved to be of little value, but a satisfactory kill of earwigs was given by sacking soaked in 3 % benzene hexachloride (Liquid Agrocide III diluted to 1 in 8). Application of the BHC was repeated at intervals of 3 weeks.

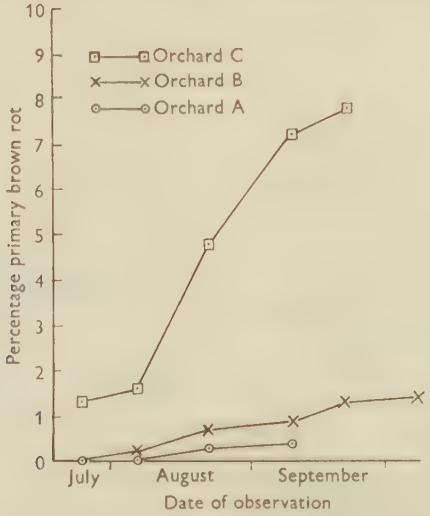
Results

Field trials, 1948 *and* 1949

The records from the preliminary investigations of 1948 are illustrated in Text-fig. 1. The conspicuous feature was the high level of brown-rot infection in orchard C, which had the smallest crop. Codling damage was negligible throughout; *Tortrix* damage occurred occasionally, but large numbers of earwigs were seen in all four orchards.

In 1949 records were made at weekly intervals in orchards A, B1 and C. Comparable trees in each orchard were used to evaluate the effect, on losses caused by brown rot, of banding with BHC-soaked sacking. The banding did not completely exclude earwigs because of the presence of branches touching the ground or interlacing with adjacent trees. There may also have been some falling off in the effectiveness of the BHC after the first 2 weeks following each application.

In 1949 there were heavy crops of fruit in orchards B 1 and C, but in orchard A the crop was lighter than the previous year. The percentage primary brown rot was considerably higher in all three orchards than in 1948 (Table 1). Text-figs. 2–4 show the progress of the disease, which was similar to that in the previous year. Particularly in orchards A and B there tended to be rapid increase in brown rot between the end of August and picking time. In orchard C the disease appeared earlier on

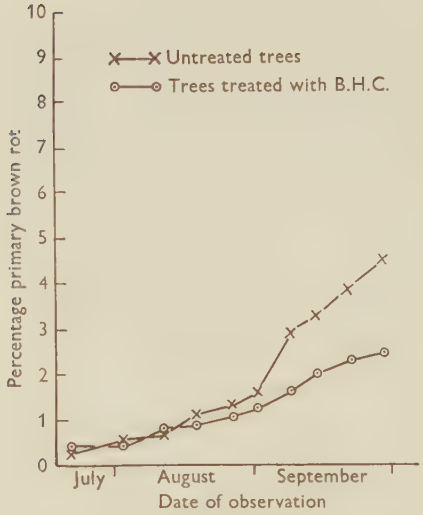


Text-fig. 1. The development of primary brown rot in three orchards, 1948.

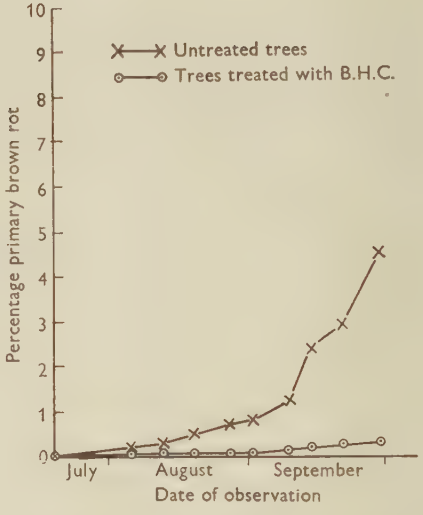
Table 1. *Numbers of apples with primary brown rot on branches of six treated and six untreated trees*, 1949

Orchard	Treated with BHC	Untreated	S.E.	Diff. required for significance 5 %	1 %
A	49	91	9·84	31·02	44·10
B	11	123	44·82	141·3	—
C	144	364	64·02	201·9	287·2
Total A+B+C	204	578	81·18	234·0	314·6

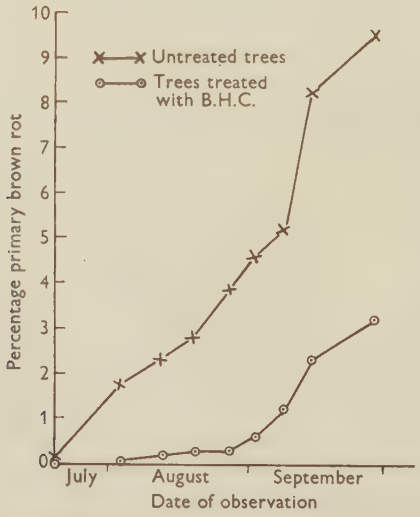
the untreated trees, apparently because of cracking of fruit on two of the trees brought about by drought conditions and also to the presence of *Botrytis* eye rot. The text-figures and Table 2 show the reduction in brown rot brought about by banding. There was considerable variation in the amount of brown rot from tree to tree, so that at centres A and C the differences due to treatment were statistically significant only at the 5 % point. If, however, the results from the three orchards are considered together the difference between brown rot on banded and unbanded trees is highly significant. Codling damage was again very slight in all three orchards. The shallow, round holes noted in previous seasons were even more conspicuous



Text-fig. 2. The development of primary brown rot on treated and untreated trees in orchard A, 1949.



Text-fig. 3. The development of primary brown rot on treated and untreated trees in orchard B, 1949.



Text-fig. 4. The development of primary brown rot on treated and untreated trees in orchard C, 1949.

at each centre. Again, many earwigs were present and up to 150 adults were trapped in each band.

Field trials, 1950

In 1950, centre A was selected for detailed recording as it carried a moderately heavy crop. The trees at this centre were fairly upright, and the weight of fruit seldom brought branches down to the ground. Ten pairs of trees were chosen, and on 19 June one tree of each pair was banded with sacking soaked in 10% BHC. The bands and the bases of the trees were dusted with 5% BHC at weekly intervals during August. The progress of the disease on treated and untreated trees is shown in Text-fig. 5, and the results summarized in Table 1. On the untreated trees 7% of the apples were infected with primary brown rot and only 2·15% on the treated, the difference being highly significant. Codling moth damage was again slight and *Tortrix* injury was also negligible, the predominant injury being holes similar to those caused by earwigs in the laboratory (see below). These holes were first noted in late July and became more common in August. On neighbouring trees of Cox's Orange Pippin similar holes were not seen until towards the end of August but became increasingly noticeable from then onwards. Sometimes fruit was damaged in orchard boxes after picking. In spite of the sacking bands earwigs were still present in some of the apple clusters. On untreated trees immature earwigs were present in large numbers from June onwards and adults were found until late October. They were usually clustered among the dense foliage and within the fruit trusses. During this period adult earwigs were always to be found at the base of the trees or sheltering under grass and in the crevices of orchard boxes. At the end of the season the sacking bands were removed from the treated trees and examined. There was in each band an average of 332 dead earwigs and three codling moth larvae.

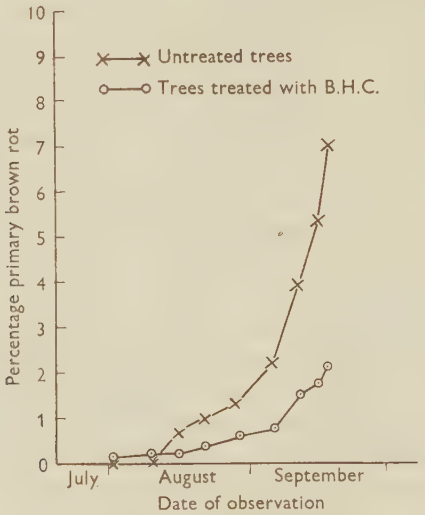
Laboratory experiments

Following preliminary indications in 1948, further observations were made in 1949 to test the ability of earwigs to damage sound fruit. In a laboratory experiment, thirty earwigs were confined in glass vessels into which were successively put apparently intact fruit of Laxton's Superb, James Grieve, Cox's Orange Pippin, Lord Lambourne and King of the Pippins. In each variety shallow, rounded holes were made. Freshly picked fruits were more readily damaged than those which had been picked for several days. Out of nineteen fruits, two had holes on the cheeks, two had holes on both the calyx end and the cheeks, three at the stalk end only and twelve at the calyx end. The observations were repeated in 1950 with similar results. Holes again were usually circular, about 4–6 mm. in diameter, with a shallow cavity hollowed out beneath the skin (Pl. 25).

Fruit survey, 1950

Having obtained the evidence that earwigs could be an important precursor of brown rot in the orchards investigated, it was decided to make a survey to see if the

problem was present elsewhere. Visits were made in late September and October to orchards in Worcestershire, Warwickshire and Herefordshire. Random samples of 500 apples of either Cox's Orange Pippin or Laxton's Superb from twenty-three orchards were taken from orchard boxes before grading. Any apple showing a blemish which caused a break in the skin was regarded as damaged, and an attempt



Text-fig. 5. Development of primary brown rot on treated and untreated trees in orchard A, 1950.

Table 2. *Primary brown rot on branches of ten treated and ten untreated trees*, 1950

	Treated with BHC	Untreated	S.E.	Diff. required for significance 5 %	Diff. required for significance 1 %
Total no. of apples with primary brown rot	43	140	12·2	31·5	55·9
Apples with brown rot as % of apples present in July	2·15	7·0	0·61	1·58	2·80

was made to classify this damage. The different categories of damage were slug and snail, earwig, codling moth, *Tortrix*, other insects, scab (*Venturia inaequalis*), cracking due to weather conditions and/or spray injury, removal of stalks when picking, and mechanical damage caused mainly by bruising or stalk punctures or abrasions by twigs. The number of apples with brown-rot infection was also counted.

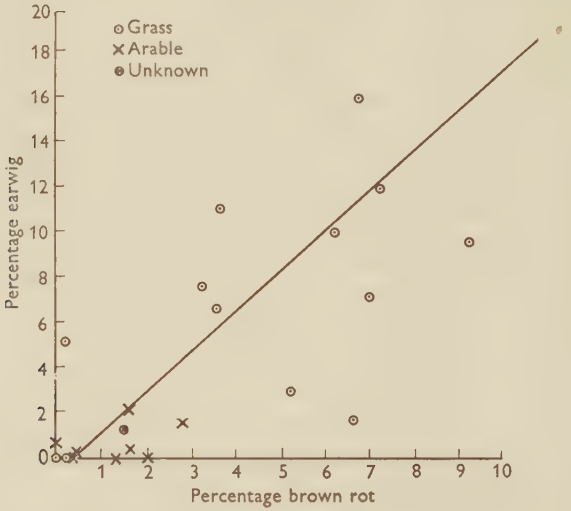
The results of the survey are summarized in Table 3. In eight of the twenty-three samples earwig damage was the most important single factor. In addition, there was appreciable earwig damage in two other cases, 5·2 % in Laxton's Superb

TABLE 3. *Survey of apples in West Midlands, 1950*

Orchard	Grass or arable	Variety	Days from picking	% damage due to various causes: Earwig	Slug	Codling	Tortrix	Other insects	Bird	Scab	Cracking	Stalk removed	Mechanical	Unknown	Total brown rot
Evesham I	G	Cox	6	16·0 (6·6)	0	2·0 (0·2)	0·8	1·2	0	2·4	0	0	1·4	0	6·8
Stratford I	G	Laxton	10	8·8 (3·4)	0	2·4	2·0	1·2	0	3·6 (0·6)	0	0·8 (0·2)	1·4 (0·2)	4·8 (4·8)	9·2
Stratford II	G	Laxton	0	5·2	0	11·4	0·4	0·2	1·2	9·6	0	3·0	1·0	0·2 (0·2)	0·2
Worcester I	G	Laxton	5	6·6 (1·8)	0·6	5·0 (0·2)	2·4	0·4 (0·2)	0	0·2	0	2·6	5·4 (1·2)	0·2 (0·2)	3·6
Worcester II	G	Laxton	5	0	0·2	2·4	0·6	0·2	0·2	0·2	1·6	1·2	5·0	0·4	0
Evesham III	G	Cox	4	10·0 (1·4)	0	5·2 (0·2)	0·8 (0·2)	1·6 (0·4)	0	24·2 (2·0)	1·5	2·6 (0·2)	2·8 (0·2)	2·2 (1·4)	6·2
Coventry	G	Cox	1	7·6 (3·0)	0	1·0	1·3	2·0	0	5·0	0	0	1·0	0	3·3
Colwall I	G	Cox	10	1·6	0·8 (0·2)	2·8 (0·6)	0·4	1·2	0·6	4·2 (0·4)	15·2 (2·6)	3·8 (0·2)	7·6 (0·2)	4·0 (1·4)	6·6
Bidford I	G	Cox	7	12·0 (3·0)	0·6	0·2	1·6	0	0	1·6	0	0·2	2·6 (0·2)	1·8 (1·6)	7·2
Bidford I	G	Laxton	7	11·1 (1·9)	0·9	0·0	5·7	2·8 (0·4)	0	0·9	0	0·4	11·0 (1·4)	0·4	3·7
Bromsgrove II	G	Cox	2	0	0	0	0	0·2	0	0·8	5·4	2·8	1·8 (0·2)	0·2	0·2
Bidford II	G	Cox	2	7·2 (1·2)	1·8	0	1·4	0·6 (0·4)	0	0·4	5·8 (1·0)	2·8 (0·2)	2·2 (0·6)	4·0 (3·6)	7·0
Bidford II	G	Laxton	2	3·0 (0·6)	0·2	0·2	1·2	0	0	0·0	2·0 (0·2)	4·2	5·1	6·2 (4·4)	5·2
Worcester III	?	Laxton	2	1·0 (0·2)	0	2·2	1·6	0·4	0	1·8	2·4	1·4	4·0	1·8 (1·4)	1·6
Worcester III	A	Cox	2	1·6	0·4	1·2	2·2	0·6	0	3·8	1·0	0·8	3·8 (0·4)	3·0 (2·0)	2·8
Stratford III	A	Laxton	8	0·2	1·2	2·4	1·2	0	0	0·2	0	4·6	2·6	0·6 (0·6)	0·6
Evesham II	A	Cox	14	0·4	1·4	0·4	1·4	0·6	0	0·6	1·0	0·4	6·6	0·6 (0·4)	1·6
Evesham II	A	Laxton	14	0	3·0	6·0	1·3	0·3	0	0	0·3	0·6	3·3	0·6 (0·6)	1·3
Ross I	A	Cox	7	0·6	0·8	0·4	1·0	1·8	0	2·8	1·8	2·0	5·7	0·4	0
Colwall II	A	Cox	10	2·2	0	0·2	0·4	1·4 (0·2)	0	2·2 (0·2)	3·0	2·2	2·2	1·0 (1·0)	1·6
Ross II	A	Cox	5	0	0·6	0·8	0	0	0	29·4 (1·6)	2·4 (0·2)	3·2	1·2	0·4 (0·2)	2·0
Bromsgrove I	A	Laxton	4?	0	0·5	0·5	0	0·5	0	0·5	13·7 (0·2)	2·2	1·5	0	0·2
Bromsgrove I	A	Cox	4?	0·2	0·5 (0·2)	0	0·2	0	0	0·7	10·0	1·0	0·7	0·5 (0·2)	0·5

Figures in brackets are percentage infected apples.

at Stratford II and 3 % in the same variety at Bidford II. In Text-fig. 6 the percentage of earwigs is plotted against percentage brown rot and the regression line fitted. The correlation coefficient was 0·79, which is highly significant. This is in spite of the fact that there was considerable variation in the time between picking and examination of different samples. In one instance, Stratford II, earwig damage was 5·2 % and there was little brown rot, but here the fruit was picked and examined on the same day. In only three cases in which there was a substantial amount of



Text-fig. 6. Regression of % brown rot on % earwig in apple samples, 1950.

brown rot did the damage caused by any other factor exceed that ascribed to earwigs. At Bidford II mechanical damage was 4·1 % and injury due to stalk removal 4·2 %, but there was also 3 % earwig damage, and in the majority of diseased apples the rot had gone so far that it was impossible to determine the precursor. At Colwall I cracking and mechanical damage seemed to be the important factors leading to brown rot. In one instance, at Evesham III, scab was important but earwig damage was also high. In three cases only was codling injury in any way important. In two of these, Worcester I and Evesham III, it was less than the amount of earwig damage, and in the third the amount of brown rot was negligible.

After the samples had been examined inquiries were made as to whether fruit came from grass or arable orchards. Earwig damage was negligible in all the fruit from arable orchards, but was considerable in all but three of the samples from grass orchards. In one of these three (Bromsgrove II) the post-blossom spray programme included three application of Parathion, while in another (Worcester I) there was a BHC sawfly spray followed by lead arsenate. It seems possible that

these measures might have successfully eliminated any earwigs that were present. In the third case (Colwall I) the most important precursors of brown rot were probably weather damage, mechanical injury and apple scab. The results obtained in the survey indicated that the amount of brown rot present in a sample was partly dependent upon the time that had elapsed between picking and examining the fruit.

Effect of sorting fruit after picking

Samples of approximately 40 lb. weight of Laxton's Superb apples were examined carefully, and any fruit with broken skin was removed. This sorting was carried out in samples on the day of picking, after 3, 7 and 14 days, while one sample was left untouched as a control. After 64 days the percentage of brown rot present in the samples was respectively 0·5, 2·3, 3·7, 19·6 and 24·4. After a fortnight the increase in brown rot was relatively slight. This is not surprising, as the apples were stored in a shed under very cool conditions and observation on stored apples by Kidd & Beaumont (1924) and Wilkinson (1938) have shown that in cold stores brown rot is not an important cause of loss. These results show the importance of removing damaged fruit as quickly as possible after picking. If fruit cannot be sorted immediately after picking it would probably pay growers to put it immediately into cold or gas store.

Discussion

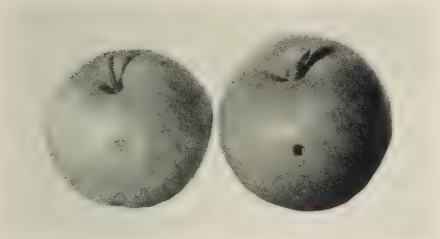
The preliminary 1947 survey of apple orchards in the West Midlands showed how difficult it was to obtain any accurate assessment of losses due to brown rot. The presence of rotting fruit on the ground cannot be taken as a clear indication that brown rot is epidemic. Any fruit damaged by falling may become infected with the brown-rot fungus. The disease develops over a comparatively long period, so that it is essential that a sequence of observations should be made on the same trees. Frequent observations on whole trees did not provide exact records, and it was necessary to examine a small proportion of the apples and to note individual infection. As labelling all the infected apples individually was not possible the only practical means was to remove and count them as soon as seen. This method had the disadvantage of cutting down the losses due to spread by contact to adjacent fruits. It did, however, give some measure of primary infection which is most important, as obviously the amount of secondary spread by contact will be related to the amount of primary infection. However, difficulty was experienced in counting the number of apples on a given branch. On some branches the numbers were very high, and it was difficult either to count the apples accurately or to observe the individual apples with certainty at later examinations. This difficulty was overcome in the 1950 season by making observations on a limited number of apples (25) on each of eight branches. This covered a large part of the circumference of the tree and in practice was comparatively easy. This method, however, is subject to the disadvantage that the number of apples under observation tends to fall due to

causes other than brown rot, but it does serve as a basis of comparison between different trees in an orchard. If comparisons are to be made between different orchards or between different seasons it seems desirable that allowances should also be made for variations in the apple population of the tree. This might be expressed as the number of apples per foot of branch under observation.

The original intention of these observations was to explore the development and intensity of brown rot in the variety Laxton's Superb under different conditions of management. However, the early observations indicated that damage of fruit by a then unknown agent seemed to be the overriding factor in the orchards studied. This damage was shown during the course of the investigations to be due to earwigs. While earwigs have at times been suspected of causing primary damage to fruit (Bush, 1943) there is no certain previous record of their doing so. However, the type of damage obtained on unblemished fruit in the laboratory agreed in all respects with that seen in the orchard. It appeared that dessert apples were attacked only as they neared ripeness, and it may be this factor coupled with the nocturnal feeding habits of the insect which has led other investigators to assume that earwigs would only feed on fruits first damaged by other agents. Furthermore, as brown rot usually follows rapidly after injury, attacked fruits are often not seen until they reach a stage of breakdown, when it is impossible to distinguish the initial injury.

In the 1950 extensive survey it appeared that earwigs were mainly associated with grass orchards. Under arable conditions cultivations may disturb developing colonies while grass would provide protective cover. It is possible that the pest may have become of increasing importance since the recent increase in the practice of grassing down. While the method of banding trees with sacks soaked in insecticide was fairly effective for the purpose of the observations this would prove cumbersome in a large orchard and other methods of control should be sought. Spraying or dusting the ground beneath the trees with BHC in early summer is likely to be promising and avoids much of the harm to beneficial insects that may ensue if trees are sprayed with persistent insecticides at that time. Although earwig damage does not occur until late in the summer the insects were present all the year round in the orchards under observation.

Bennett, Kearns & Marsh (1944) refer to a plot of cordon apples in which about 10 % brown rot was present although codling moth was virtually absent. As their records were made only at picking time this must underestimate the total loss incurred. Similarly, Moore (1950*a*, *b*) showed that in a trial plot exhibiting a good control of codling moth, sawfly and apple scab, 10 % brown rot was present. Our observations indicate that although the amount of brown rot varies from site to site and season to season under West Midland conditions, part of this loss may be due to earwig damage. The 1950 survey showed that this source of injury to fruit was present in a number of widely scattered orchards in Worcestershire and Warwickshire, being most prevalent in grass orchards. Whether earwig



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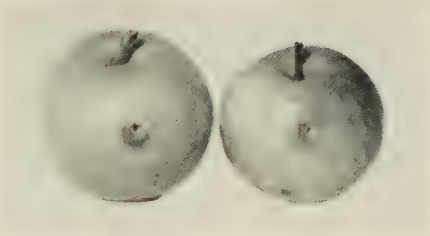

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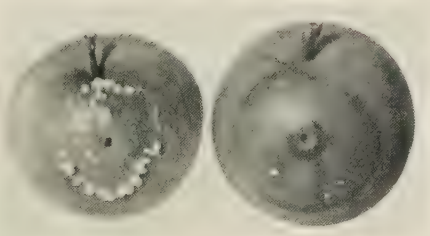

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B.

CROXALL, COLLINGWOOD AND JENKINS—*Observations on brown rot of apples*

damage has an even wider distribution or if its occurrence was due to unusual seasonal conditions can only be determined by more observations in future years.

We wish to thank all those growers who allowed us to examine their apples and who showed such interest in the investigation. We are particularly grateful to Mr Mills, Fruit Manager of S. F. Blackwell, Ltd., Messrs A. H. and R. Hiller of Dunnington Fruit Farm, Mr D. W. Fitzgerald and his foreman, Mr Farley, for providing plots for the experimental work and also for making many valuable suggestions. Our thanks are also due to Miss C. F. Ganderton, who helped to make many of the counts in the field.

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EXPLANATION OF PLATE 25

A. Stages in development of brown rot following earwig damage. Dates of photographing: (1) 3. ix. 51; (2) 4. ix. 51; (3) 5. ix. 51; (4) 7. ix. 51.

B. Apple cut to show typical earwig damage.

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THE INFLUENCE OF *NOSEMA APIS* ON THE LARVAL HONEYBEE

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In experiments on the infection of bee larvae with *Nosema apis*, the parasitic spores did not germinate and infected adults did not result. In hives of bees infected with *N. apis* about 10–20 % of the eggs laid did not complete their development probably because of inadequate care and feeding.

The effect of *Nosema apis* on the larval honeybee

The silkworm moth, *Bombyx mori*, is sometimes attacked by a parasitic protozoan, *Nosema bombycis*, which is closely related to *N. apis*. It has been shown by Pasteur (1870), Stempell (1909) and Kudo (1924) that the larval silkworm can become infected with *N. bombycis*, and it was therefore thought that *N. apis* might also attack the larvae of its host, the honeybee. Experiments were made to test this hypothesis.

Method

Two brood combs containing many eggs and young larvae were removed from a healthy colony of honeybees. A portion of one of these combs (comb A), containing several hundred young larvae about 24 hr. old, was selected, and a drop of syrup containing viable spores of *N. apis* was mixed with the brood-food surrounding each larva. As soon as the larvae had been fed in this way, the comb containing them was placed in the brood nest of a colony of bees known to be suffering from Nosema disease. A group of larvae of the same age in the second comb (comb B) was similarly treated, but instead of returning this comb to the hive immediately, it was kept in an incubator at 33° C. for 12 hr. before being placed in the brood nest of a colony suffering from Nosema disease. It was hoped that this would ensure that the larvae fed on some of the contaminated syrup.

Two or three days after inoculation larval bees selected at random were removed from comb A and sectioned.

A few of the larvae in comb B were also taken, their alimentary canals removed, macerated on a slide, and examined for the presence of the spores of *N. apis*. The remainder of the larvae were allowed to develop normally, and a number of the resulting pupae and adults were examined in order to determine whether or not they were infected with *N. apis*.

Results

Examination of transverse and longitudinal sections of the larvae removed from comb A showed the presence of spores of *N. apis* in the ventriculi, but none of these spores was found to have germinated.

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The results of examination of pupal and adult bees derived from the experimentally infected larvae in comb B are shown in Table 1.

The results show that none of the pupae or adult bees contained spores of *N. apis*. Thus *N. apis* differs from *N. bombycis* in its mode of attack on its host.

TABLE 1. *Results of attempted infection of pupal and adult bees via their larvae*

Date on which examined	Pupae			Imagos		
	No. examined	No. infected	No. not infected	No. examined	No. infected	No. not infected
9. ix. 48	16	0	16	20	0	20
13. ix. 48	15	0	15	25	0	25
14. ix. 48	20	0	20	19	0	19
15. ix. 48	18	0	18	20	0	20
16. ix. 48	—	—	—	20	0	20

THE INFLUENCE OF *NOSEMA APIS* ON THE VIABILITY OF THE EGGS AND LARVAE OF THE HONEYBEE

It has been observed that the sealed brood produced in colonies of bees known to be suffering from Nosema disease is frequently of a 'spotty' nature, many empty cells being interspersed amongst normal capped cells containing pupae which ultimately give rise to adults. It was decided to study the viability of the eggs laid in colonies infected with *N. apis* at different times of the year.

Method

Four colonies of bees known to be infected with *N. apis* and three healthy colonies of bees were selected in the same apiary. At intervals during the season brood-combs were removed from each of these colonies, the adult bees brushed off, and records made of those cells that contained eggs. The combs were then replaced in the colonies from which they had been taken. About 13 days later the same combs were removed once more, the cells in which eggs had been observed on the first examination relocated and a note made whether these cells now contained fully grown larvae or pupae.

The position of between 200 and 300 cells which contained eggs was noted in the combs of each colony at the time of the first examination.

Results

The results obtained in these investigations are given in Table 2. The fact that a larva was found in a cell at the time of the second examination does not necessarily mean that this larva was derived from the egg observed at the time of the first examination, since when an egg fails to hatch the worker bees will frequently remove it and the queen may lay another egg in its place. None the less the figures given are substantially correct.

It is clear that a fairly high proportion of the eggs laid by the queen of a colony that is suffering from Nosema disease fail to give rise to pupae. Some of the eggs in

such colonies either fail to hatch or, if they do hatch, a number of the resulting larvae die at an early stage. Since it has already been shown that the larva of the honeybee does not become infected by *N. apis*, it is concluded that these eggs died because they were faulty, or because they were not given adequate care by infected worker bees. The deaths of very young larvae were most probably caused by inadequate feeding by infected worker bees whose pharyngeal salivary glands were not secreting normally, or by feeding with insufficient or poor-quality food produced by the

TABLE 2. *Showing the percentage of the eggs which failed to give rise to full-grown larvae or pupae in four colonies of bees infected with* Nosema apis, *and three healthy colonies at different times of the year*

	Colonies infected with *N. apis*								Healthy, control colonies					
	Colony B1		Colony B2		Colony B3		Colony B4		Colony A1		Colony A2		Colony A3	
Date of examination	% adult pop. infected	% eggs failed	% adult pop. infected	% eggs failed	% adult pop. infected	% eggs failed	% adult pop. infected	% eggs failed	% adult pop. infected	% eggs failed	% adult pop. infected	% eggs failed	% adult pop. infected	% eggs failed
11. iv. 49	30	17	32	15	30	9	36	17	0	1	0	1	0	2
26. iv. 49	48	25	40	26	38	19	38	15	0	0	0	0	0	0
4. v. 49	58	21	54	32	62	24	58	22	0	1	0	3	0	2
31. v. 49	60	28	60	21	50	21	40	18	0	0	0	0	0	0
13. vi. 49	48	18	44	14	20	14	30	10	0	0	0	0	0	1
26. vi. 49	50	19	20	11	10	11	24	12	0	3	0	0	0	2
22. vii. 49	18	6	12	6	10	7	10	3	0	0	0	1	0	0
31. vii. 49	10	3	10	5	16	9	16	7	0	1	0	4	0	0
% eggs which failed to give rise to sealed brood during the period of examination		17·1		16·2		14·3		13·0		0·4		1·1		0·9

healthy worker bees of the colony which attempt to feed an abnormally large number of larvae because of the inability of their infected sisters to do so. The latter explanation would appear to be the more probable since early infection with *N. apis* inhibits, or at least greatly reduces, the quantity of brood-food secreted by the bees concerned, and also tends to cause them to join the foraging population of their colony at an abnormally early age.

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OBSERVATIONS ON THE LIFE HISTORY AND BIOLOGY OF *TIPULA LATERALIS* MEIG.

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(With 7 Text-figures)

The occurrence of large numbers of larvae of *Tipula lateralis* Meig. in watercress beds has not hitherto been recorded. Observations on commercial beds and laboratory experimental work show this species to be primarily a saprophyte feeding on rotting submerged cress leaves, though it can develop during the final instar on green cress. Populations as high as 250,000/acre do not affect cress beds adversely.

There are two generations a year and probably a partial third. The life cycle can be completed in 63 days. Mating and oviposition are described, the preferred site for egg laying being on wet soil. The average number of eggs is over 500. The egg and first-instar larva are described for the first time. There are four instars, the anal segment of the first being radically different from that of the remainder. It has been shown that pupation cannot take place under water as previously stated.

In March 1948 large numbers of a leatherjacket appeared in certain watercress beds in south Lincolnshire. This leatherjacket, which was reported to be causing damage by biting through the stems of the cress, occurred most commonly around the concrete sides of the beds, although some were found among the plant stems. Specimens collected on 20 March and placed on damp moss began pupating on 27 March, and the first imago emerged on 3 April. This species was identified as *Tipula lateralis* Meig.

Mating and oviposition

Both male and female are ready for mating and pair freely immediately after drying off. If breeding jars were emptied of adults late at night, all the freshly emerged flies were usually paired by the next morning. If left undisturbed *in copula* both males and females die within 24 hr., but if separated after half an hour they remain alive for several days and the female lays eggs that are almost all fertile.

As *T. lateralis* is an aquatic or semi-aquatic species, tests were made to ascertain the type of substratum best suited for oviposition. A tray 3 ft. × 1 ft. 6 in. × 4 in. deep was filled with dry soil and the surface made uneven with a depression running the length of the tray where the soil was only 1 in. deep. The tray was then watered well and allowed partially to dry until the sides of the tray were dry while the soil in the trough remained almost waterlogged. Females enclosed in a cage over this soil laid their eggs on the moist soil only.

While laying eggs the female stands vertically on her third pair of legs with wings vibrating rapidly, but passing through only a small arc. She raises and lowers

herself so that the tip of the ovipositor is touching ground-level or is about $\frac{1}{4}$ in. above. These raising movements are rather irregular, but are carried out, on an average, twice a second. At intervals she curls the abdomen forward slightly, so that the ovipositor is at an angle to the level of the soil, comes down rather more heavily, and forces the ovipositor in about $\frac{1}{8}$ in. At each of these heavier thrusts an egg is laid, slightly below the surface of the wet soil. Eggs were seen to be laid at intervals of approximately 2 sec.

This procedure, first seen in the laboratory, was later observed on a watercress bed on 15 April and on many subsequent occasions under warm sunny conditions. The bed had been cleared of cress and the water was being drained off. Some parts of the soil were uncovered, but there were many small streamlets still trickling through the bed. The flies were laying eggs on the tops of small mounds in the uneven sludge surface. Their activities could be checked by the appearance of many small pinpricks upon an otherwise smooth wet surface. The female while dancing in this way was constantly moving slowly backwards and forwards over the area.

In the laboratory, oviposition took place in the small box in which mating had taken place. Eggs were laid more readily if three or four layers of moist filter-paper covered the bottom of the box, and the filter-paper facilitated the removal and counting of the eggs.

Number of eggs laid and period of egg-laying

Detailed observations were made on eight ovipositing females. The numbers of eggs laid were 585, 491, 642, 425, 629, 637, 254 and 423 respectively, averaging 511. Barnes (1937), studying *T. paludosa*, records 200 as the average for that species under laboratory conditions with a potential egg-laying of 270.

In the laboratory, egg-laying was spread over a period of 4 or 5 days. The maximum life of flies so confined in small boxes, with no food but plenty of moisture, was 6 days. Some flies oviposited the same day as emerging and mating, but most were observed to have about a 24 hr. break before beginning to lay.

The egg

The egg (Fig. 1 *a*) is asymmetrical in shape, rather more pointed at the anterior than the posterior end. One side is almost straight and the other is considerably curved. The length varies between 0·78 and 0·72 mm. with an average of 0·75 mm., the maximum width observed was 0·26 mm., the minimum 0·21 mm. and the average of fifty eggs 0·245 mm.

As with other *Tipula* spp., the egg is jet black, glistening, with neither reticulation nor sculpturing. The micropyle is prominent, and is situated on the line of greatest curve, approximately one-tenth of the length of that side from the anterior end. It is placed in the centre of a cup-like depression, the edge of which is somewhat raised above the general level of the surface. A long-drawn-out filament, very fine

and delicate but extremely tough, arises from the posterior end of each egg. The individual filaments from a batch of eggs laid together coalesce to form one rather stouter thread, so that when one egg is lifted by means of a needle, twenty or thirty attached eggs are often seen to be hanging below the needle.

These filaments, which have not previously been recorded on the eggs of *Tipula* spp., are probably a means of anchoring the eggs, causing them to be entangled among weeds, etc., if they are disturbed by a rise in the level of water. According to Miall (1912), Grenacher (1868) described somewhat similar anchoring threads attached to the eggs of *Ephemera vulgata*.

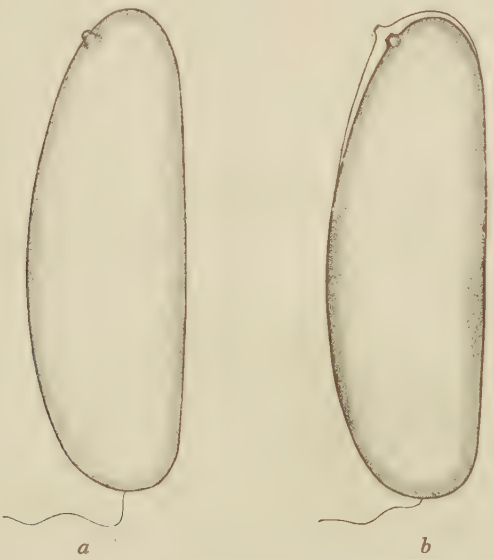


Fig. 1. *a*, egg of *Tipula lateralis*; *b*, empty egg shell.

Emergence from the egg

The larva emerges through a slit made in the egg shell (Fig. 1 *b*) which is regular in position and size. The slit is along the line of greatest curve of the egg passing through the micropyle. It stretches downwards to approximately one-third of the length of the egg and upwards from the micropyle over the anterior end and downwards on the opposite side for a short way. The regularity of this split is most noticeable, and the many empty eggs examined all conformed to this type.

Incubation period and percentage hatching

The incubation period lasts 6–7 days, as compared with about 14 days for *Tipula paludosa* (Barnes 1937).

After oviposition, the eggs were separated and placed twenty at a time on moist filter-paper in a Petri dish. From a series of twenty such dishes put up on 13 April,

Table 1 shows a series of hatchings which took place. Thus 63 % of the eggs hatched after 6 days, with a few more (3 %) on the following day. No further hatching occurred. During the second generation eggs laid on 16 June hatched on the evening of 22 June. Later in the year, third generation eggs laid on 9 September hatched on 22 September—a longer period.

Table 1

Dish no.	No. hatched after 6 days	No. hatched after 7 days
1	10	—
2	13	—
3	12	2
4	14	1
5	16	—
6	10	—
7	14	1
8	12	1
9	16	—
10	18	1
11	12	—
12	13	—
13	13	2
14	13	—
15	8	1
16	10	1
17	8	2
18	14	—
19	13	—
20	14	1
Total	253	13

The rearing of the larval instars and experiments on feeding

For breeding experiments it was necessary to obtain a suitable food plant on which to feed the developing larvae. Barnes (1937), rearing *T. paludosa* Meig., had used germinating wheat, chickweed, clover, cabbage leaf, slices of potato and bran. Of these, germinating wheat, potato and bran were tried without success, the larvae refusing to feed. When watercress was tried, the yellow rotting leaves were taken readily, but the roots and the green aerial leaves were refused. The green cress was eaten by larvae in the final instar, but the yellow was preferred; at no time was the root tissue or stems eaten. In all rearing experiments, and at all stages, the normal food provided was this yellow decaying cress.

The larvae were kept in groups in 9 cm. Petri dishes, being cleaned out on average three times a week. Some were kept separately and cleaned out each day to ascertain the number of ecdyses and the dates on which they occurred. It was essential at all times to maintain a reasonable amount of water in the dishes—usually about 2 mm. in depth, as *T. lateralis* is very susceptible to drying out.

The life history of *Tipula lateralis*

There are four instars—the history of typical larvae can be seen from the data in Table 2.

Table 2

	May	May	May	May	May
Egg laid	14	14	14	14	14
Egg hatched	20	20	20	20	20
First moult	30	28	30	29	28
	June	June	June	June	June
Second moult	10	10	8	9	6
Third moult	23	24	17	20	22
	July	July	July	July	July
Pupated	12	9	7	9	10
Emerged	19	16	15	17	17

After moulting, the larva eats the cast skin, leaving only the head capsule. Hence, the appearance of a cast head capsule is the only reliable indication of moulting, though there is also normally a noticeable increase in size.

The average times for the various stages were:

Egg	6 days
First instar	9 days
Second instar	10 days
Third instar	13 days
Fourth instar	18 days
Pupa	7 days
Total	63 days

Description of the larval instars

Three previous workers (Beling, 1878; Lévy, 1920; Oldham, 1929) have described the final instar and pupa, but none have reared the insect through from egg to adult, consequently the first instar is dealt with more fully than the later instars which have been adequately described.

First instar larva

On emergence from the egg, ten first instar larvae had the measurements shown in Table 3.

The larva is light grey to cream without the longitudinal dorsal markings which are characteristic of the final instar. The integument is semi-transparent, the gut contents and tracheal system being easily discerned; it is covered with a dense fine pubescence.

It is almost cylindrical in shape, being only slightly flattened dorso-ventrally. It is widest across the thorax, slightly tapering to the anterior and posterior ends. The head can be completely retracted into the first thoracic segment.

The posterior end (Figs. 2, 3 and 4) has an upper stigmatic area and a lower anal area. The *stigmatic area* has two pairs of protuberances, a ventral and a lateral pair, the former being somewhat larger. Each protuberance is bluntly triangular in shape, curving over gently outwards and backwards. The inner side of each process has a more acutely triangular area of a dark brown colour extending the full distance from base to apex. In addition, the ventral pair of protuberances have a single stout bristle on the inner side near the apex. The stigmata are placed towards the distal end of, and above, the lateral processes, being separated by approximately $1\frac{1}{2}$ times their own diameter. They are not flush with the surface but slightly convex. The cup-shaped stigmatic chamber can be readily seen owing to the transparence of the integument. This stigmatic area folds gently over to join

Table 3

	Length (mm.)	Breadth (mm.)
a	1·83	0·409
b	1·65	0·383
c	1·77	0·400
d	1·93	0·333
e	1·60	0·367
f	1·82	0·283
g	1·75	0·302
h	1·87	0·433
i	1·77	0·381
j	1·83	0·417
Average	1·78	0·370

imperceptibly the dorsal surface of the larva, and is slightly indented. Along this fold there are eight groups of bristles which are constant in number and position. The outer groups are placed outside and above the stigmata. The next two groups are above the outer and inner edges of the stigmata respectively, and the inner pair are found nearer to the dorso-central line of the body. Each group consists of a small papilla on which are either two or three bristles. The number varies with the individual, but the outermost groups on each side more consistently have two bristles than the inner two pairs. These setae are all directed forwards at a very acute angle over the dorsal surface of the larva, so that they point in the same direction as the bristles on the ventral processes. When the larva breaks the surface of the water at an angle, the bristles, together with the surface tension of the water film, cause the stigmata to be open to the atmosphere though below the level of the water surface.

The gentle indentation at the centre of the junction of the stigmatic area with the dorsal surface is of interest, as in all later instars two more processes are developed one on each side of this area, making a ring of six. In the first instar only are there four, the dorsal pair being represented by the groups of bristles.

The *anal area* is convex, with the anus in the middle. At the margins of the anal area are four conical fleshy outgrowths which act both as gills and organs of

Fig. 2. *Tipula lateralis*: anal segment of first-instar larva; lateral view.

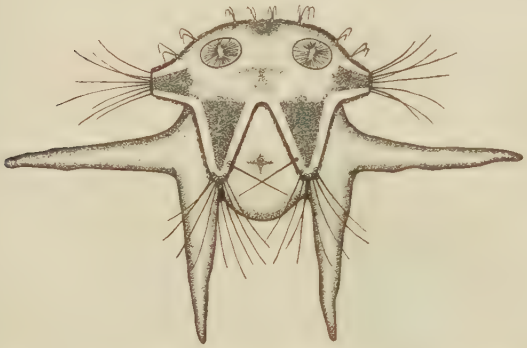


Fig. 3. *Tipula lateralis*: first-instar larva, hind view.

Fig. 4. *Tipula lateralis*: first-instar larva, dorsal view.

locomotion. The upper pair are directed outwards, the lower pair downwards, their average length being 0·13 and 0·10 mm. respectively. This area, like the stigmatic area, produces in later instars a further pair of processes which are directed backwards and serve as an additional pair of gills.

Second instar larva

This differs appreciably from the first instar, primarily because of the development of an extra pair of anal gills, and the replacement of the groups of bristles on the dorsal surface of the stigmatic area by a further pair of protuberances.

Morphologically, the second- and third-instar larvae appear to be identical with the fully developed larva as described by Oldham (1929). At the beginning of this instar the length averages 5·5 mm. and the breadth 1·0 mm. It is no longer semi-translucent, but is cream to grey in colour and has traces of the dark mid-dorsal stripe which is characteristic of the later instars.

Third instar larva

Immediately after ecdysis, this instar averages 9·5 mm. in length and 1·6 mm. maximum breadth. Morphologically it is akin to the second and fourth instars, but it is darker than the former, yet not as deeply coloured as the final instar larva.

Fourth instar larva

Oldham's (1929) description of this, the full-grown larva, needs no amplification except in one respect. Oldham, quoting Alexander (1920), considers that in the tipulid larvae with six gills the posterior branches of the posterior gills are generally atrophied. The three pairs of gills present are the anterior bifid pair and the posterior pair, of which the posterior bifurcation has atrophied. Considering that the first-instar larva has only two pairs of gills, it is doubtful whether *T. lateralis* has developed from a larva with eight gills; instead, the six-gill stage has here been reached because of the bifurcation of one pair, rather than the bifurcation of both pairs and the subsequent atrophy of one branch of the posterior gill. Examination of numerous specimens of fourth instar larvae (Figs. 5, 6) suggests that the backwardly directed smaller gills are not, as Oldham considers, the basal branch of the anterior pair, but rather the anterior branch of the posterior pair.

THE PUPA

The pupa (Fig. 7) has been described by Beling (1878) and Lévy (1920). The sexes are easily distinguished by an examination of the last abdominal segment. In general, the present observations confirm the descriptions of the above writers, but several small points not previously mentioned are worthy of note. In the male, the outermost pair of legs are never quite the same length as the other pairs.

Beling (1878) considers that the pupa seldom has more than sixteen bristles or

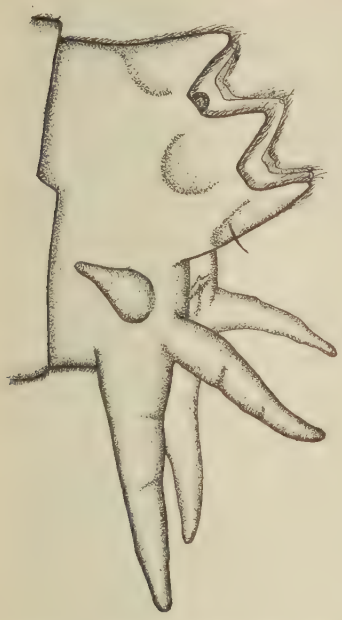


Fig. 5. *Tipula lateralis*: anal segment of fourth-instar larva; lateral view.

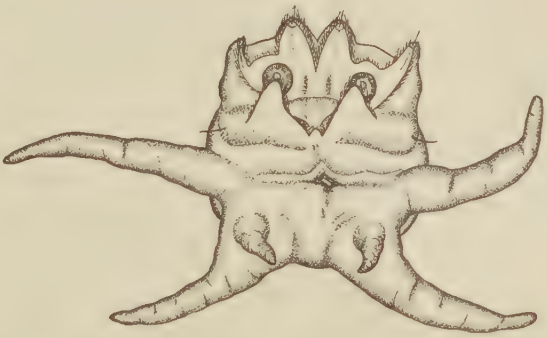
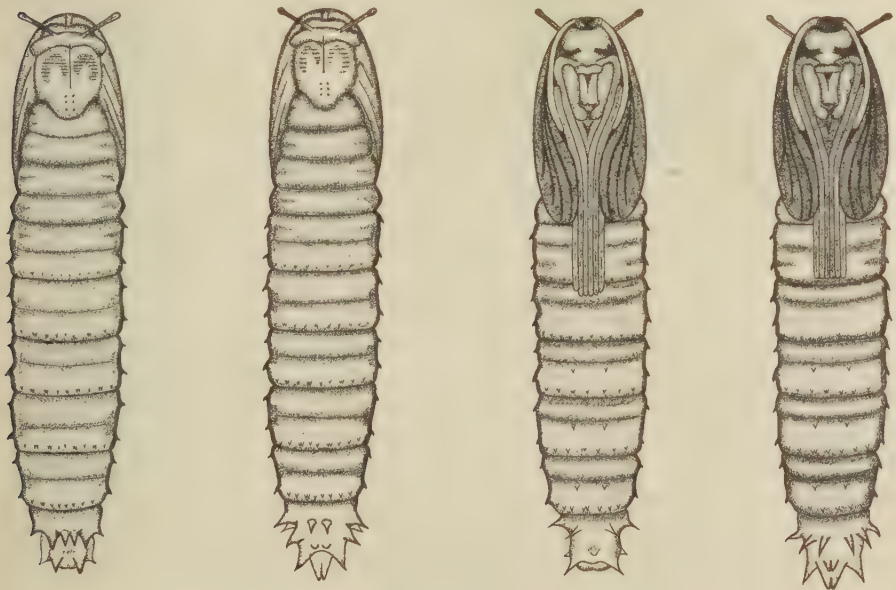


Fig. 6. *Tipula lateralis*: anal segment of fourth-instar larva; hind view.



♂ dorsal ♀ dorsal ♂ ventral ♀ ventral.

Fig. 7. Pupae.

'thorns' making up the ring on the posterior edge of each abdominal segment, whereas all the specimens now examined had not less than sixteen, there commonly being as many as twenty-two. The number varies, but Beling's maximum of sixteen is too low.

Both male and female have on the ventral surface of the fifth segment four small bristles. These appear to be more regular in size, shape and position than the larger bristles comprising the rings on lower segments, and have been overlooked by both Beling and Lévy.

Site for pupation

In the laboratory efforts were made to ascertain the conditions best suited for pupation. To do this a series of large glass cells were made up containing soil with various amounts of water.

Five such cells were arranged containing: (*a*) dry soil, (*b*) moist soil, (*c*) very wet soil, (*d*) waterlogged soil with a film of surface water and (*e*) soil completely covered by half inch of standing water. Into each of these were introduced sixty full-grown larvae. It was seen that the larvae in (*e*) did not pupate and eventually died. After 1 month the emergences were: (*a*) 45, (*b*) 33, (*c*) 42, (*d*) 14, (*e*) 0. This indicated that *T. lateralis* does not pupate under water or at least very seldom. This is in agreement with Grünberg (1910), but contrary to the observations of Gerke, quoted by Grünberg, who stated that pupation may occur in the water should the larva be prevented from reaching land. Continuous search in watercress beds has failed to show any pupae below water-level.

It is very noticeable that in cress beds the fully fed larvae make towards the sides of the beds, and then crawl out of the water in search of suitable sites. Where the sides of the beds are earthen, the larvae burrow into the soil above water-level and pupate. If the sides are of concrete they pupate either in cracks and crannies or crawl away from the bed in search of suitable conditions. *T. lateralis* will pupate in a variety of media. It has been found under sods, under the iron rails of the miniature rail system surrounding some cress beds, under moss and lichen. On 3 April 1950, pupae were collected at the rate of 500 per hour per 50 yards from the porous soft upper inch of brickwork surrounding a cress bed.

Populations in cress beds

No satisfactory method has been found for estimating populations in growing cress, but some observations have been made on the soil prior to planting the crop. Square-yard areas were treated with orthodichlorobenzene and counts made of the larvae coming to the surface. This method worked admirably either on smooth dry soil or soil that had recently had water running over it. It was shown that the infestation was very variable over the beds, with only a slight tendency to higher populations at the lower end of the beds. For example, on one bed 7 sq.yd. areas were treated on 24 May 1949, and the numbers of larvae found were 0, 33, 17, 0, 40 and 25. Forty larvae per sq.yd. is equivalent to 193,600/acre.

Although *T. lateralis* is considered an aquatic or semi-aquatic species, drying and cultivation does not provide control. A bed giving an average population of 300,000/acre had been previously drained and baked dry, with cracks $\frac{1}{2}$ in. wide, ploughed and harrowed.

Number of generations

Under laboratory conditions there are three generations per year. Under natural conditions it is rather difficult to be certain of the number as there is considerable overlapping during the summer months.

The winter is spent in the larval stage, and feeding is completed by the end of March to early April, with the appearance of adults during April. This emergence, the first generation of the year, is the only clear-cut mass emergence. Owing to the varying time of development of the larvae, second generation flies have been found from the first week of June onwards, with indications of a peak around the second or third week of June. Observations over several years have shown adult *T. lateralis* to be on the wing at all times from June till late October. The latest date recorded was 24 November 1950, when a solitary female was observed egg-laying on a slight mound in a cress bed in north Lincolnshire. It is apparent that there are two generations, with a possibility of at least a partial third.

Economic position of *Tipula lateralis* in watercress beds

When larvae of this species were found in abundance in watercress beds, the growers, bearing in mind damage done by terrestrial leatherjackets, e.g. *T. paludosa* Meig., considered that these too must be harmful pests. The early feeding experiments, culminating in the choice of decaying cress leaves as food, suggested that the growers' fears were unfounded. Nevertheless, cress beds were carefully watched, but no case was seen where *T. lateralis* could be associated with failed patches.

In the spring of 1950 a small area 6×4 ft. on a cress bed at Tetney, north Lincolnshire, was netted with butter muslin, and 200 pupae of *T. lateralis* introduced. The hatch of adult flies was satisfactory, and large numbers of larvae were produced, but the cress grew quite well, and could not be seen to be different from the cress growing outside the cage.

As the experiment took place during April and May, the months of optimum growth of cress, it was decided to repeat the experiment in a modified form. In October 1950 a small area of cress was blocked off from the main cress bed, having covered entry and exit for water, but preventing the escape of larvae, and 487 small larvae introduced. The size of the experimental plot was 10 sq.yd., giving a population of approximately 250,000/acre. Young larvae were feeding throughout the winter months during a time when the growth of watercress is almost at a standstill. Even so, no damage was apparent, and the cress within the experimental area was as thick and healthy as that outside.

It is concluded therefore that larvae of *T. lateralis* are saprophytic feeders, and at present cause no damage in watercress beds. However, the occurrence of this

insect in cress beds appears to be a relatively recent development, no previous record being found in literature, and as insects can readily change their habitat, their food and feeding habits, further observations on *T. lateralis* are desirable.

Grateful acknowledgement is made to Mr A. Roebuck for his valuable help and suggestions, to watercress growers in Lincolnshire for the facilities afforded, and to Miss J. Beaumont, who helped with collecting and with the breeding and rearing in the laboratory.

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MUSTARD OILS AND CONTROL OF THE POTATO-ROOT EELWORM, *HETERODERA ROSTOCHIENSIS* WOLLENWEBER: FURTHER FIELD AND LABORATORY EXPERIMENTS

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(With 1 Text-figure)

Allyl isothiocyanate has already been shown to be effective against the potato-root eelworm. Experiments are described in which this mustard oil, which occurs in the seed of black mustard, is compared with two similar substances; one, phenyl isothiocyanate, is synthetic, the other, phenethyl isothiocyanate, occurs in the roots of many crucifers, including black mustard.

In hatching experiments, various concentrations of each of these oils in potato-root excretions are used to stimulate eelworm cysts. The earlier results with allyl isothiocyanate are confirmed; it is also shown that the emergence of larvae decreases with increasing concentration of oil. An experiment in which different concentrations of oil are used in different strengths of root excretions showed that it is unlikely that there is any stoichiometric interaction between root excretions and mustard oil.

Field experiments were carried out in different years and on different plots so that their comparison is difficult. But they appeared to show that talc is inferior to peat as a carrier for mustard oil. A comparison of the three oils on talc gave no significant improvement in yield, although plants treated with the allyl oil did best. In another trial with allyl isothiocyanate on talc, highly significant improvements in yield were obtained at dosages of 8 and 12 g. per row of ten plants, but the improvements were smaller than those reported previously for oil on peat. In a peat experiment on a plot giving the poor control yield of 4 oz. a plant, yield increased regularly with dosage and was almost trebled at 9 c.c. of oil per row of ten plants. On a plot with control yields of over 20 oz. a plant, 6 c.c. of oil per row increased yield by over 50%. It is shown that the increased yields are almost certainly due to a reduction in the severity of the attack, i.e. that the laboratory hatching trials 'explain' the field results. Although the cyst counts reported are by no means beyond criticism, they show conclusively that treated plants producing heavier crops of potatoes bear fewer cysts than control plants with lower yields.

There is a suggestion that polyploidy may be induced by certain dosages of allyl isothiocyanate.

Introduction

In 1925, Morgan found that white mustard seedlings grown together with a potato plant in a pot of infected soil reduced the severity of eelworm attack. Triffitt (1929, 1930) then showed that potato-root leachings no longer stimulated eelworm emergence if mixed with leachings from the roots of white mustard seedlings, but the use of white mustard plants as green manure was unsuccessful as a control measure (O'Brien & Prentice, 1930, 1931; MacM. 1932). Later (Ellenby, 1945*a*, *b*),

it was shown that black mustard seedlings possessed the same property, and further, that dilute solutions of allyl isothiocyanate, the mustard oil of black mustard seed, were also effective. A small-scale field trial in which mustard oil was applied on peat to the drills at the time of planting gave increases in yield of about 100 % with dressings equivalent to about 0·1 cwt. of oil per acre. The present paper describes attempts to extend these findings.

Hatching experiments

The mustard oil used in the earlier trials was the allyl form which is derived from the glucoside sinigrin present in black mustard seed; white mustard seed, on the other hand, contains sinalbin, which yields the mustard oil *p*-hydroxylbenzyl isothiocyanate. The work of Stahman, Link & Walker (1943) has shown, however, that the roots of these and of many other crucifers, contain neither of these oils but phenethyl isothiocyanate. It was clearly desirable that the activity of this substance should be compared with that of the allyl form; moreover, in view of the work of Smedley with phenyl isothiocyanate (1939) which I have discussed elsewhere (1945*a*), it was decided to test this substance also.

Hatching experiments were carried out in 1945 and 1946, in which the different oils were compared. Another experiment was run at the same time as the first of these series, which was designed to throw light on the mode of action of the allyl form; for convenience this will be described later.

Hatching experiment, 1945; allyl, phenyl and phenethyl isothiocyanates

In previous experiments (Ellenby, 1945*a*) solutions of allyl isothiocyanate at dilutions 1/2000, 1/20,000, and 1/1,000,000 in potato-root excretions had been tested. The strongest of these solutions killed the eggs, while 1/20,000 reduced emergence very considerably without causing permanent injury. As a first step, it was decided to compare the oils on a 'molar' basis, and at the strengths 0·00001, 0·00002, 0·0001 and 0·0005 M; for allyl isothiocyanate, the strongest and weakest of these solutions corresponded almost exactly to 1/20,000 and 1/1,000,000, the former of which was so effective in the earlier experiment.

Each oil was made up as an 0·01 M solution by dissolving the appropriate amount in 10 c.c. of alcohol; this was then added to a large bulk of distilled water and made up to a litre. Standardized solutions of potato-root excretions were prepared from Great Scot plants throughout the experiment by a method described elsewhere (Ellenby, 1944*b*). These solutions were used to prepare the appropriate dilutions of the mustard oils made up so that the strength of root excretion was identical in all; a control series with added alcohol contained no mustard oil.

No attempt was made to buffer the solutions against change in pH as it was considered undesirable to introduce factors which might interact differently with the various oils. In any case, tests with a glass electrode had shown that the oils had very little effect on pH; as shown in Table 1, there is a slight tendency in all

cases for the oils to increase acidity, but there is very little change with concentration and, at any concentration, the hydrogen-ion concentrations are not very different. As solutions of root excretions have pH's within the same range, it is unlikely that, at the dilutions employed, the pH of the oil-poor-excretion mixtures would be any different.

Each solution was tested at room temperature on cysts obtained by flotation from the air-dried soil of an infected plot in lots of twenty-five, using the single-cyst technique already described (Ellenby, 1943). Larvae were counted and removed almost every day, and freshly prepared solutions of a similar sort added to all cells

TABLE 1. pH *of solutions of three mustard oils*

Concentration	Allyl	Phenyl	Phenethyl
0·00001 M	7·02	6·50	6·83
0·00002 M	7·00	6·61	6·80
0·0001 M	6·74	6·68	6·52
0·0005 M	6·68	6·50	6·50

Distilled water: 7·02

every 4 days until, on addition of fresh solution, very little emergence ensued. This was about a month from the beginning of the experiment. All solutions were then removed, and, over a period of 3 days, each cyst was subjected to four changes of distilled water in an effort to remove all traces of the mustard oils. Finally, root excretions from the last control lots and of appropriate strength were substituted and larvae again counted and removed as they emerged.

Results 1

After the month's experimentation with the different solutions, emergence in root excretion alone was very low and has been neglected in the treatment of the results; presumably the root excretions had already lost their effectiveness. During the course of the experiment it was noticed that certain of the cysts were slightly broken; results from these cysts were excluded, as, in general, emergence from broken cysts is considerably higher than that from normal cysts (Ellenby, 1946).

Because a skewed distribution is given by the values for larval emergence per cyst, a logarithmic transformation is used in the analysis of the data (Ellenby, 1944*b*); $\log(x+1)$ is used, rather than $\log x$, the number of larvae, in order to avoid difficulties with cysts producing no larvae. Mean values of $\log(x+1)$ for the different solutions, essentially the logs of the geometric means, are shown in Table 2. There are some notable differences among the values.

The results of an analysis of variance gave a value for F, the variance ratio, of 3·16; with 289 degrees of freedom, this shows highly significant effects of treatments ($P<0{\cdot}01$). Standard errors vary between $\pm 0{\cdot}122$ and $\pm 0{\cdot}136$, and the S.E. for the difference between two means between $\pm 0{\cdot}175$ and $\pm 0{\cdot}187$, depending on the number of cysts in the groups. But only in the allyl isothiocyanate group is there any suggestion of a tendency for emergence to fall with increasing strength of oil,

with mean values for log $(x+1)$ of 1·485, 1·004, 1·147 and 0·873 at the four strengths of oils. However, with a standard error of ±0·175, only the last of these values shows a significant difference from control ($P=0·02$) at a strength roughly equal to the 1/20,000 which gave successful results in previous trials (Ellenby, 1945*a*). Phenyl isothiocyanate, if anything, shows a trend in the opposite direction, that is, emergence seems to increase with the strength of oil used; but none of the values differs significantly from control. In the case of phenethyl isothiocyanate, both 0·00002 and 0·0005 M solutions reduce the emergence almost equally and significantly ($P=0·05$); but, paradoxically, the intermediate treatment 0·0001 M does not differ significantly from control.

TABLE 2. *Emergence of larvae from cysts treated with mixtures of potato-root excretions and three mustard oils*, 1945. *Mean values of log* $(x+1)$, *where* $x=$ *larvae emerging per cyst, and* S.E.*

Concentration (M)	Potato-root excretions in mustard oils		
	Allyl	Phenyl	Phenethyl
0·00001	1·485 ± 0·128	1·188 ± 0·128	1·154 ± 0·128
0·00002	1·004 ± 0·128	1·315 ± 0·132	0·894 ± 0·139
0·0001	1·147 ± 0·136	1·507 ± 0·124	1·460 ± 0·124
0·0005	0·873 ± 0·124	1·320 ± 0·128	0·891 ± 0·132

Control, root excretions alone: 1·283 ± 0·129

* Based on error mean square.

Hatching experiment, 1946*; allyl and phenyl isothiocyanate*

The 1945 experiment showed that certain dilutions of both allyl and phenethyl isothiocyanates are effective in reducing larval emergence in the presence of potato-root excretions. On the other hand, there was no reduction in the case of phenyl isothiocyanate which, if anything, seemed to give an increased emergence; it seemed desirable to repeat this part of the experiment.

There were a number of differences from the technique employed in 1945. The actual test solutions were prepared from a solution ten times more dilute, as considerable difficulty had been experienced in getting the oils, particularly the phenyl isothiocyanate, into solution. No attempt was made to standardize the root excretions used. They were obtained by standing the whole root system of an actively growing plant in water for 2 hr. These solutions were used in preparing two series of mustard oil solutions, allyl and phenyl, each at the same series of dilutions used in the previous trials, viz. 0·00001, 0·00002, 0·0001 and 0·0005 M; the strength of root excretion was the same in all. The cysts used in this experiment were obtained from the roots of Arran Banner plants grown the previous year. Each solution was tested on twenty-five cysts, using a single-cyst technique, and controls were provided by two lots of twenty-five cysts in root excretion of comparable strength, and two lots of twenty-five cysts in distilled water. The experiment was carried out at a constant temperature of 23° C.

As before, larvae were counted and removed almost every day, and freshly prepared solutions supplied to all cysts every 4 days until after about a month emergence had practically ceased. Cysts were then soaked in three changes of distilled water over a period of 3 days as before, and root excretion of appropriate dilution of the last series added. This was ineffective, as in the last experiment; accordingly, freshly prepared excretions were added without dilution, and at 4-day intervals, until after about another month emergence had again practically ceased.

Results 2

Mean values for log $(x+1)$ during stimulation with the mustard oils are presented in Table 3, column A. The mean value for the three weaker allyl isothiocyanate solutions are all similar and somewhat lower than controls in root excretions; as in

TABLE 3. *Emergence of larvae from cysts treated first with mixtures of potato-root excretions and allyl or phenyl isothiocyanates (A), and then with potato-root excretions alone, 1946. Mean values of log $(x+1)$ per cyst; values in column B are for total emergence in both phases of the experiment*

Solution in first phase; all in root excretions in second phase	Concentration (M)	Mean values of log $(x+1)$ A	B
Root excretions in allyl isothiocyanate	0·00001	1·256	1·872
	0·00002	1·226	1·698
	0·0001	1·268	1·660
	0·0005	0·945	1·696
Root excretions in phenyl isothiocyanate	0·00001	1·098	1·623
	0·00002	1·299	1·674
	0·0001	1·416	1·759
	0·0005	0·542	1·025
Root excretions alone		(i) 1·536	1·813
		(ii) 1·457	1·682
Distilled water		(i) 0·318	1·309
		(ii) 0·401	1·268
S.E. of any mean value		±0·141	±0·123

previous experiments, emergence in the 0·0005 M solution in root excretion is considerably lower than control. The results with phenyl isothiocyanate are again somewhat puzzling. Emergence increases with the strength of oil used, from a mean value of 1·098 in the 0·00001 M solution, to 1·299 in 0·0002 M, and 1·416 in 0·0001 M; however, in the strongest solution, 0·0005 M, emergence is very low indeed at 0·542. Analysis of variance gives a value for F, the variance ratio, of 8·95, showing highly significant effects of treatments; this is not surprising, as the treatments include water alone. However, the mean square for error is ±0·494, giving a S.E. of any mean value of ±0·141 and a S.E. of any difference of ±0·2. Differences in emergence between both 0·0005 M allyl, phenyl isothiocyanate, and excretion control are highly significant ($P<0·01$ and $<0·001$ respectively), and emergence in 0·00001 M phenyl isothiocyanate is also significantly lower than control ($P=0·05$).

Moreover, emergence in 0·0005 M phenyl isothiocyanate does not differ significantly from emergence in distilled water.

If the analysis is pushed a step further and different treatments grouped under oils, it is found that total emergence for either allyl or phenyl isothiocyanates, all strengths, is significantly different from excretion controls ($P < 0{\cdot}01$ in both cases).

After the various cyst groups had been stimulated with the different solutions, they were all stimulated with a strong solution of root excretion until all emergence ceased. In Table 3, column B, mean values for total emergence for each group are shown; these values are based on emergence in both phases of the experiment. Values are remarkably similar in all cases except for those that had been stimulated with 0·0005 M phenyl isothiocyanate in the first phase of the experiment, and the values for the two lots which had spent the first phase in distilled water; all these values are low, the first, at 1·025, very low indeed. Analysis of variance gave a variance ratio of 4·2 showing significant effect of treatments. The S.E. for any mean is $\pm 0{\cdot}123$, giving a S.E. for the difference between any two means of $\pm 0{\cdot}174$. Total emergence from the cysts of lot 0·0005 M phenyl isothiocyanate is therefore significantly less than that from the cysts in root excretion alone throughout the experiment (P almost 0·001). It is curious, however, that cysts which had been in distilled water in the first phase also show significantly lower total emergence than cysts in excretions (P about 0·01); it will be remembered that the experiment was continued until emergence had practically ceased.

Root-excretion—mustard-oil hatching experiment, 1945

Mustard oil must either act on potato-root excretion itself or on the eelworm; it was hoped that at least the former possibility could be tested. Essentially the experiment consisted of a series of tests in which different dilutions of the mustard oil were tested in different dilutions of root excretions, i.e. at any particular strength of either oil or excretion there were a number of dilutions of either excretion or oil. If there were a simple interaction between root excretion and mustard oil the experiment would be an attempt to 'titrate' root excretions with mustard oil, using the eelworm as an indicator!

Root excretions were prepared as already described for the 1945 experiment. With these, a series of solutions of allyl isothiocyanate was prepared at the strengths 0·00001, 0·00002, 0·0001 and 0·0005 M; this composed series X. A second series of oil dilutions was made up in which the strength of root excretions was half that of the previous series, giving series Y. In the third series, the strength of root excretions was halved again to give series Z. There were thus three comparable series of dilutions of mustard oil which differed only in the strength of root excretions in which they were made up. Controls were provided by three lots of root excretions of strengths X, Y and Z, similar to those of the series X, Y and Z respectively. Each solution was tested on twenty-five cysts. The full-strength, X series, formed part of the 1945 experiment described above in which the three

different mustard oils were compared; this experiment was carried out in the same way, freshly prepared solutions being added at 4-day intervals until all emergence had practically ceased.

Results 3

Mean values for log $(x+1)$ are presented in Table 4 for the fifteen solutions under test. At each strength of root excretion, mean values tend to fall progressively with increasing strength of mustard oil. This trend is most marked in the half-strength root excretion series, Y, with values 1·207 for control, and 0·926, 0·735, 0·558 and 0·315 for increasing strengths of mustard oil. Emergence is low in all solutions in the most dilute root-excretion series, so that there is little scope for any

TABLE 4. *Root-excretions—mustard-oil trial*, 1945. *Emergence of larvae from cysts treated with solutions of potato-root excretions of different strengths in solutions of allyl isothiocyanate of different concentrations. Mean values of log* $(x+1)$, *per cyst, and* S.E.

Solution	Concentration of mustard oil (M)	Mean values of log $(x+1)$ for excretion-strengths X, Y and Z* X	Y	Z	Means across excretion-strengths and S.E.
Root excretion alone	—	1·283	1·207	0·893	1·130 ± 0·083
Allyl isothiocyanate	0·00001	1·485	0·926	0·447	0·967 ± 0·083
	0·00002	1·004	0·735	0·484	0·734 ± 0·083
	0·0001	1·147	0·558	0·220	0·631 ± 0·083
	0·0005	0·873	0·315	0·339	0·514 ± 0·083
Means across oil-strengths and S.E.		1·165 ± 0·107	0·752 ± 0·107	0·475 ± 0·107	

* Z is the weakest solution of root excretions, Y is twice as strong as Z, and X twice as strong as Y.

trend, although there is some; but the fact that the trend is not so marked in the case of the strongest root-excretion series suggests that the action of the oil depends on the strength of stimulation with root excretion.

At each strength of mustard oil values fall with dilution of root excretion. Summing across oil strengths gives mean values of 1·165, 0·752 and 0·475 for the three strengths of root excretion. Summing across excretion strengths gives mean values of 1·130 for control, and 0·967, 0·734, 0·631 and 0·514 for increasing concentration of oil. It is interesting that the increase from 0·00001 to 0·00002 M effects a greater reduction in emergence, 0·967–0·734, than the much greater remaining increases. Analysis of variance, analysing for both oil strengths and excretion strengths, gives variance ratios of 21·6 for excretions, and 6·8 for oil strengths; both values are highly significant, the former notably so.

It is most striking that the sets of values for emergence in the various mustard-oil solutions keep apart. In spite of the fact that the strongest mustard-oil solution is fifty times as strong as the weakest, while the weakest root excretion is only a quarter of the strength of the strongest, there is no overlap in the values for emergence in

the root-excretion sets. For example, the geometric mean for emergence from cysts treated with 0·00001 M mustard oil in strongest root excretion is 29·6; a fiftyfold increase in mustard-oil strength reduces the value to 6·5, a value similar to that obtained by a twofold dilution of root excretion. It is interesting, however, that the value for emergence in full-strength excretion in 0·00002 M mustard oil is very similar indeed to the value for half-strength excretion in 0·00001 M oil, for these two solutions are in some senses 'equivalent'.

The overall results show the general trend more clearly. Geometric mean values for emergence obtained by summing across oil strengths are 13·6, 4·65 and 1·98, i.e. halving the strength of root excretion reduces the emergence by almost a third, and a further halving halves emergence almost exactly. On the other hand, values obtained by summing across excretion strengths show that doubling the concentration of oil reduces the geometric mean from 8·27 to 4·42, say by half, after which there is little change, a fivefold increase in oil concentration reducing the mean value to 3·28, and a further fivefold increase to 3·27. Clearly there is little basis for maintaining that there is any simple stoichiometric reaction between root excretion and mustard oil.

FIELD EXPERIMENTS

1945

The results of a small-scale field trial with allyl isothiocyanate have already been described (Ellenby, 1945*b*). A small dressing of allyl isothiocyanate, equivalent to about 0·1 cwt. per acre applied on peat to the drill at the time of planting, increased the yield by about 100 %; but as control yields varied along the length of the plot used in the randomized block experiment from about 4 oz. per plant to over 10, doubling the poor control yields still gave a poor yield. The 1945 experiment attempted to discover whether increased dosages of mustard oil would increase such poor yields.

The experiment was carried out on the poor half of the plot used in the earlier experiment. There was thus room for only two blocks, each of four rows of plants, set out at right angles to those of the previous trial, so that the treatments of the previous year would be evenly distributed. Allyl isothiocyanate was applied on peat at three rates, viz. 3, 6 and 9 c.c. per ten plants, each quantity being stirred up in a gallon of water and thoroughly mixed with about 4 lb. of peat which was sufficient to absorb it. Each dosage was therefore tested on a single row of ten plants in each of the blocks, and in each case a control row was treated with water and peat.

The various mixtures were added to the drills at the time of planting, and Great Scot potato setts placed directly on top of the peat at 1 ft. intervals in the rows; drills were 3 ft. 6 in. apart to avoid interaction of the treatments. Each row also received a light dressing of a balanced fertilizer at the rate of 1 oz. per yard of drill. The crop was harvested when the last plant had died down and yields measured to the nearest ounce per plant.

Results 4

Total yields, in oz. per row, are presented in Table 5; in Fig. 1 treatment means are plotted against dosage. The success of the treatments is well shown.

Yields in block 2 increase steadily with dosage from a control yield of 7·7 oz. per plant to 18·5 at 9 c.c. per row, an increase of 240 %. Even more striking, however, are the yields in block 1, which coincided more or less with the worst section of the

TABLE 5. *Field trial*, 1945. *Allyl isothiocyanate applied on peat at three rates. Total yields per row of ten plants* (*oz.*)

Mustard oil per row (c.c.)	Blocks 1	Blocks 2	Treatment means and S.E.
0	40	77	58·5 ± 6·4
3	78	123	100·5 ± 6·4
6	93	164	128·5 ± 6·4
9	112	185	148·5 ± 6·4

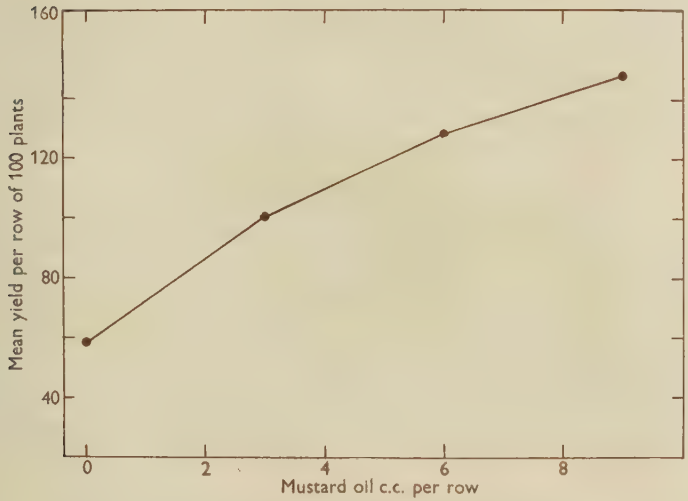

Fig. 1. Increase in yield with dosage of allyl isothiocyanate.

earlier experiment. Here the control yield is only 4 oz. per plant; it is almost doubled in the 3 c.c. row at 7·8 oz. as in the earlier experiment, increases again in the 6 c.c. row, and is almost trebled in the 9 c.c. row, where the yield is raised to the almost respectable level of 11·2 oz. per plant.

An analysis of variance showed significant effects of both blocks and treatments. The S.E. of any mean total yield is equal to ± 6·4 oz., which gives a value of ± 9·07 oz. for the S.E. of the difference between any two mean total yields. All differences from control are significant, *P*, the probability, being equal to 0·02 in the case of the smallest difference from control.

1946, 1947 *and* 1948

The experiments described above or elsewhere (Ellenby, 1945*b*) have shown, then, that allyl isothiocyanate, applied on peat to the drills at the time of planting, increases the yield very considerably; laboratory tests (Ellenby, 1945*a*, and above) suggested that this effect was due to reduced emergence of larvae. It seemed desirable to consider the following points: (1) Could similar effects be obtained with other mustard oils, such as the naturally occurring phenethyl isothiocyanate or the synthetic phenyl isothiocyanate? (2) Could the oil be applied with the same effect on a carrier more convenient than peat? (3) Was the increased yield due to a smaller eelworm attack? The answers to these questions were the objects of the field trials carried out in 1946, 1947 and 1948. They could have been sought more effectively in a single experiment of appropriate design; unfortunately, this was impossible with the facilities at my disposal.

The experiments were carried out on infested allotments, each year's experiment on a different plot of about the same dimensions. They were all laid out as randomized block experiments, with four blocks of four treatments in 1946 and 1947, and three blocks of four treatments in 1948; each treatment was represented by a single row of ten plants in each block. Great Scot seed was always used, set a foot apart, with 3 ft. 6 in. between rows to avoid interaction of treatments. As in previous experiments, each row received a light dressing of a balanced fertilizer.

1946. *Allyl, phenyl, and phenethyl isothiocyanates*

Oils were absorbed on talc and compared on a molar basis as in the hatching trials described above. In the case of allyl isothiocyanate, 6 g. of the oil was thoroughly mixed with 8 oz. of Canadian talc and applied to a row of ten plants. As the specific gravity of this oil is almost exactly 1, this application is about equal to the middle dosage rate of the previous experiments. Comparative molar dosages of the other oils were similarly applied, viz. 8·2 g. of phenyl and 10·4 g. of phenethyl isothiocyanates, per row, each on 8 oz. of talc. The control rows received talc alone. As before all dressings were applied to the bottom of the drill at the time of planting.

Results 5

During growth control plants appeared to be doing less well than the others; this impression was confirmed by the yields, presented in Table 6, which are lowest

TABLE 6. *Field trial*, 1946. *Allyl, phenyl and phenethyl isothiocyanates applied on talc at equimolecular concentrations. Total yield per row of ten plants* (*oz.*)

	Blocks				
Treatment	1	2	3	4	Treatment means and S.E.
Control	94	146	91	49	95 ± 12·96
Allyl	160	142	116	80	124 ± 12·96
Phenyl	118	140	72	70	100 ± 12·96
Phenethyl	102	117	162	74	114 ± 12·96

for controls in most cases. Nevertheless, there is very little improvement with treatment, the mean values for total yield per row being little greater than the control yield of 95 oz., with allyl highest at 124·5 oz. and phenyl lowest of the treatments. An analysis of variance showed significant effect of blocks, but not of treatments, for which the mean square is little greater than that for error. Clearly the experiment shows that at the dosages employed, talc is a less effective carrier than peat. It also appears that phenyl and phenethyl are less effective than allyl, and certainly not superior.

1947. *Allyl isothiocyanate applied on talc at three dosages*

In view of the 1946 results it seemed desirable to examine whether allyl isothiocyanate would be effective on talc at higher dosage rates. Accordingly, it was applied at the rates 4, 8 and 12 g. per row each on 8 oz. of talc. Otherwise the experiment was similar to that of the previous year.

Results 6

Six weeks after planting control plants all appeared to be poorer than the treated plants; this difference later disappeared. Yields are shown in Table 7. Control

TABLE 7. *Field trial*, 1947. *Allyl isothiocyanate applied on talc at three rates. Total yields per row of ten plants* (*oz.*)

Mustard oil per row (g.)	Blocks				Treatment means and S.E.
	1	2	3	4	
0	112	136	110	79	109·3 ± 6·5
4	128	115	133	115	122·8 ± 6·5
8	165	146	152	121	146·0 ± 6·5
12	165	133	143	110	137·8 ± 6·5

plants give the lowest yields in three out of the four blocks, and in all blocks the highest yields are given at the 8 g. dosage. An analysis of variance shows significant effects of both blocks and treatments (P almost 0·01 in both cases). The S.E. derived from the error mean square = ± 6·47 oz., giving a S.E. for the difference between any two mean total yields of 9·15. Difference from control is highly significant in the case of the 8 g. treatment, approaches this level of significance for 12 g., and is not significant in the case of the 4 g. treatment. Nevertheless, the increases in yield are comparatively modest compared with those obtained in the peat experiments.

1948. *Allyl isothiocyanate applied on peat at three dosage rates*

The small increases in yield obtained in 1947 raised doubts as to the effectiveness of mustard oil. Although the experiments were carried out with talc rather than the peat which gave the original successes, it was thought possible that the latter successes might have been helped by peculiarities in the allotment. It therefore seemed desirable to repeat the experiment with peat as a carrier.

The trial was carried out in exactly the same way as the 1945 field experiment described above, on another part of the allotment used in 1947; allyl isothiocyanate was applied at the rates 3, 6 and 9 c.c. per row of ten plants, but there were three randomized blocks of four rows of ten plants instead of the two blocks of the earlier test.

Results 7

There was a certain delay in the growth of some of the treated plants; recovery was very quick, however, except in the case of the plants treated with the heaviest dosage where very few grew at all. This treatment was excluded from the analysis of the results. Total yields for the remaining rows are presented in Table 8; they are high in all cases, control plants giving over 20 oz. per plant. In spite of these heavy control yields, yields in the rows treated with 6 c.c. of mustard oil are

TABLE 8. *Field trial*, 1948. *Allyl isothiocyanate applied on peat at two rates. Total yields per row of ten plants* (*oz.*)

Mustard oil per row (c.c.)	Blocks			Treatment means and S.E.
	1	2	3	
0	204	214	224	214·0 ± 19·5
3	209	220	313	247·3 ± 19·5
6	320	265	392	325·7 ± 19·5

considerably higher, but there is little difference for the 3 c.c. treatment. Analysis of variance shows no significant effect of blocks but a significant effect of treatments ($P < 0{\cdot}05$). The S.E. for any mean total yield is ± 19·5 oz., giving a S.E. for the difference between two means of ± 27·6; yields for the 6 c.c. treated rows therefore differ significantly from control ($P < 0{\cdot}02$).

While all rows treated with 9 c.c. of mustard oil failed, there were a few plants in each row which grew. These were of enormous size, gave large yields and showed no signs of maturing when the experiment was ended in October. It has been pointed out to me by Dr P. S. Hudson that these features suggest that the plants which grew were probably polyploids. Their yields may be of interest: in oz. for each individual plant, they were 86, 50, 78, 40, 86, 72, and 128/2 for two inseparable plants.

Cyst counts, 1947–8

Hatching experiments suggested the improvement in yield as a result of mustard oil treatment was due to a reduced emergence of larvae, but there was no evidence that this, in fact, was the case. Apart from the usual difficulties in assessing the cyst population of a given soil, it was particularly difficult to obtain information of a change as a result of a treatment from the present experiments. As each treatment was applied to a particular row of plants, separated by over a yard from the next row, changes would be exceedingly localized. The results obtained are only presented as an indication of possible trends.

No attempts were made to obtain information in the highly successful experiments of 1944 and 1945. The use of peat made cyst separation very troublesome, and as, at the time, it was thought that talc would be a successful carrier, it was decided to leave the question to a later stage; this, of course, was a mistake, and the difficulties had to be faced in 1948. The 1946 experiment in which the three oils were applied on talc showed no significant improvement in yield; it was therefore not until the 1947 experiment that the question was investigated.

Soil samples were taken at random from various parts of the plot before planting. Because of the difficulties mentioned above, the technique employed in collecting soil samples after lifting was as follows. Each plant was shaken vigorously over the drill as it was lifted, so that as much as possible of the soil adhering to the root system fell off. When the whole row had been lifted, the surface soil in the line of the drill was raked over thoroughly; soil samples taken from the surface layers were used in the determination of the final cyst count. The final samples are hardly comparable with those taken before planting, but they will be comparable among themselves and thus indicate the effect of the mustard oil treatments. Cysts were obtained by flotation from 20 g. samples of air-dried soil, and three such samples were examined for each row.

Results 8

(i) 1947. Mean values for each row are presented in Table 9. The number of cysts per 20 g. sample before planting was just over seventy, so that rows show considerable increases; but the final samples were obtained from soil which had

TABLE 9. *Allyl isothiocyanate on talc at three rates. Final cyst counts from rows of 1947 field trial; mean number of cysts in 20 g. of air-dried soil*

Mustard oil per row (g.)	Block				Treatment means and S.E.
	1	2	3	4	
0	93	186	348	146	193 ± 43·1
4	248	417	256	220	285 ± 43·1
8	254	296	249	337	284 ± 43·1
12	347	262	276	164	262 ± 43·1

surrounded the roots of the plants, and they are therefore hardly comparable with the original samples. Comparing them among themselves shows that the lowest overall mean value is given by control rows, with the values for the various mustard oil treatments somewhat higher. An analysis of variance shows, however, that there are no significant effects due either to blocks or treatments. As this experiment showed significant improvements in yield in certain cases (Table 7), this fact is of some importance.

(ii) 1948. Results from the 1948 experiment are presented in Table 10. Values for control and 3 c.c. are almost equal, but that for 6 c.c. is considerably lower. An analysis of variance shows significant effects for both blocks and treatments

($P < 0.05$ in both cases) and gives a S.E. for the difference between any two means of ± 34.3; the cyst counts for the 6 c.c. treatment are therefore significantly lower than control. Again it must be pointed out that these cyst counts cannot be compared with counts based on the samples taken before planting. Nevertheless, while all final counts show increases over initial values, in the case of the 6 c.c. treatment there is little doubt that the increase is very small indeed. As this was accompanied by an increase in yield, compared with control, of well over 50 %, it is clear that the cyst data support the view that increases in yield in the field are due to the same mechanism as operates in hatching experiments in the laboratory; a reduction in the emergence of larvae reduces the severity of the attack on the plant.

TABLE 10. *Allyl isothiocyanate on peat at two rates. Final cyst counts from rows of 1948 field trial; mean number of cysts in 20 g. of air-dried soil*

Mustard oil per row (c.c.)	Blocks 1	2	3	Treatment means and S.E.
0	208	243	123	191 ± 17·2
3	229	243	110	194 ± 17·2
6	109	134	93	112 ± 17·2

DISCUSSION

Allyl isothiocyanate occurs naturally in black mustard seed. I have been given to understand, however, that it could be obtained very easily as a by-product of the refining of mineral oil so that it possesses considerable advantages over the other two mustard oils examined. Against this background, the question is not whether phenyl or phenethyl isothiocyanates are better or worse than the allyl oil but only whether they are markedly superior; this is fortunate, for, although, on the whole, allyl appears to be consistently superior, the results are by no means completely clear.

The results already published showed that allyl isothiocyanate reduced larval emergence at a dilution of 1/20,000; even a solution at 1/1,000,000 appeared to have some effect. The present results confirm these findings; emergence was reduced to about a third by a solution roughly equivalent to the former, and, although not reduced significantly at greater dilutions there was a tendency for it to decrease with increasing oil concentration. On the other hand, phenethyl isothiocyanate reduced emergence significantly at both 0·0005 and 0·00002 M; as its molecular weight is almost twice that of allyl isothiocyanate, clearly, on a weight or molar basis, it was effective at greater dilutions. However, as an intermediate dilution was ineffective, the result should not be taken too seriously; one would certainly not conclude from it alone that phenethyl is markedly superior to allyl isothiocyanate.

For phenyl isothiocyanate the results are less clear and it is possible that this oil works in a different way. At most dilutions more larvae emerged in oil root-excretion mixtures than in potato-root excretion alone so that it appeared to

stimulate, rather than repress emergence, though the differences were not statistically significant. In one series of experiments, however, a 0·0005 M solution reduced emergence to a greater extent than a similar solution of allyl isothiocyanate; indeed, emergence did not differ significantly from emergence from cysts in distilled water. Moreover, while, in general, emergence reached normal levels when cysts were transferred from the oil-root-excretion mixtures to root excretions alone, in the case of this particular solution, although emergence increased, its final level was significantly below control. However, cysts kept in distilled water for the first part of the experiment also produced significantly fewer larvae on subsequent stimulation; in fact, total emergence for the 0·0005 M phenyl isothiocyanate and distilled water cysts did not differ significantly. It is therefore difficult to assess the significance of this observation particularly as the experiment was continued until emergence had practically ceased.

While the significance of all the results of the hatching experiments is not completely clear, there are certainly no grounds for believing that either of the other mustard oils is markedly superior to the allyl form. The results of field comparison support this; although no yields differed significantly from control, the allyl isothiocyanate treatment appeared to be the most successful.

Three trials with allyl isothiocyanate on peat have now given successful results, in different years and on different plots. The experiment already reported (Ellenby, 1945*b*) showed that a dressing of 3 c.c. of oil per ten plants increased the yield by about 100 %. But doubling is not enough if control yields are very low; it is therefore of some importance that it has now been shown that yields increase with dosage. In the experiment described above, yields increased regularly with dosage and were almost trebled with a dressing of 9 c.c. per row of ten plants, equivalent at normal planting distances, to only 0·3 cwt. per acre. The 1948 control yields were high, at over 20 oz. per plant, so that one would hardly expect an improvement; nevertheless, 6 c.c. per ten plants increased yields by over 50 %.

It is difficult to compare experiments carried out under different conditions; it does seem evident, however, that although significant improvements were obtained with allyl isothiocyanate on talc, peat is a superior carrier.

In a recent paper, Johnson & Townend (1949) show that the addition of small quantities of ammonium carbonate to potato-root excretions results in a reduction in emergence; they correlate this effect with the alkalinity of the resulting solutions in which the hatching factor, or 'eclepic acid' (Calam, Todd & Waring, 1949) would be destroyed, and show that similar 'inhibitory' effects are evident in pot experiments as the pH approaches 8·0. They link these observations with experiments on the effect of partial soil sterilization, and demonstrate that hatching is delayed only so long as there is a sufficient concentration of ammonia. These important observations, clearly, do not apply to the mustard oil experiments, certainly as far as pH is concerned; none of the oils examined has much effect on the hydrogen-ion concentration; if anything, they slightly increase the acidity. It

also seems unlikely that the effect of mustard oil can be due to partial soil sterilization, for such small dressings would hardly have such lasting effects. It has been possible to show that there is no direct interaction between mustard oil and potato-root excretions; but the mechanism of its action remains obscure.

Hatching trials show, then, that mustard oil reduces emergence, and field experiments that treatment with small quantities of mustard oil increases yield; it has now been possible to demonstrate that the increased yields are almost certainly due to a reduction in the severity of the attack. A reduction at a critical early stage may still lead to an increase of the final number of eelworms, as it may enable the plant to survive a heavier subsequent attack (Peters & Fenwick, 1949). As these authors put it, 'Chitwood & Feldmesser (1948) show that there is normally a very high mortality of hatched larvae in the soil, of the order of 90 %, due to lack of available space on the roots. A fumigant killing much less than this proportion will merely facilitate the early establishment of the plant, after which the operative law is: the better the plant, the more the cysts.' Although the determinations of cyst number in the present work are by no means beyond criticism, it can be said with confidence that they show that the number of cysts does not increase with yield; that is, the better plants bear fewer cysts. It is gratifying to be able to report this breach of the law.

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A SIMPLE METHOD FOR TESTING THE TOXICITY OF VOLATILE ANTISEPTICS TO WOOD-ROTTING FUNGI

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(With 1 Text-figure)

Methods used for determining toxicity of volatile compounds to fungi are reviewed and a simple laboratory method for carrying out such tests is described.

Results from this type of test may be applicable in practice where the mycelium of the fungus is freely exposed to the action of the vapour, but if it is desired to sterilize infected wood of any appreciable thickness, information about the penetration of the vapour across the grain of the wood and of its sorption by the wood must be obtained before any treating schedule can be drawn up.

Introduction

Much work has been carried out on methods for testing the toxicity of volatile compounds to insects, but far less information is available about the toxicity of such substances to fungi. The testing of volatile compounds to wood-rotting fungi presents certain difficulties in that it is necessary to provide in the test chamber a medium on which the test fungus can be grown and its growth after treatment observed. When carrying out tests with these fungi mycelium must be used, since a regular supply of fresh spores cannot readily be obtained and the germination of the spores of many species is slow and irregular.

The nature and amount of the medium present may affect the results, depending on the rate of absorption of the chemical and upon its reaction, if any, with the medium; it is therefore necessary to standardize the amount of medium present.

Previous work

Vanine & Vladimarskaya (1933) examined the toxicity of various gases and volatile compounds to *Merulius lacrymans* and *Coniophora cerebella* growing in wood and to the spores of two common moulds. In their tests with wood-rotting fungi they exposed thin pieces of infected wood in desiccators of known volume, into which known amounts of the chemicals were introduced. After varying periods of exposure to the vapour, the viability of the fungus in the test pieces was tested by placing them on a nutrient medium and observing if further growth occurred. Acetic acid was found to be one of the most toxic to these fungi, killing *Merulius* at a concentration of 0·002 c.c./l. and *Coniophora* at 0·005 c.c./l. after 24 hr. Formaldehyde was found to be effective against *Merulius* at 0·025 c.c./l. after 22 hr. or 0·1 c.c./l. after 30 min., and against *Coniophora* at 0·025 c.c./l. after 22 hr. Pryor & Walker (1939) described a method for testing the toxicity of volatile compounds by

putting cultures, growing on 2 in. diameter disks of agar, cut from a large film of uniform thickness, on the lids of Petri dishes or one pint fruit jars with glass lids sealed down with rubber gaskets and wire clamps. By this method, Pryor, Walker & Stahlmann (1940) measured the toxicity to three species of fungi of the vapour of allyl isothiocyanate. They found that 0·05 mg./pint was sufficient to inhibit the growth of cultures of *Colletotrichum circinans*, *Aspergillus alliaceus* and *A. niger*, while 1·6 and 2·4 mg. were necessary to kill *A. alliaceus* and *A. niger* respectively.

Clark & Leonard (1947) tested the toxicity of about 250 volatile organic compounds by placing a small amount of the substance to be tested in a small cylindrical cup 1 cm. internal diameter with capacity of 0·25 ml. in the centre of a Petri dish of 10 cm. diameter, which was filled with nutrient agar inoculated with a suspension of mixed mould spores. Growth started at the edge of the plate, and the width of inhibition zone around the cup was taken as a measure of the toxicity of the compounds. The dishes were examined after incubation for 40 and 120 hr. at 30° C. The most promising substances tested were tri-cresyl phosphate and trioxymethylene. Of the thiocyanates tested, amyl-thiocyanate was found to be the most effective.

Scheffer and Duncan (1946), who exposed malt agar and samples of leather, gum, sapwood and tape to a mixed infection of moulds, concluded that benzaldehyde, 2-chloropyridine, ethyl mercuric chloride and *o*-chlorophenol were the most promising of the forty-seven compounds tested.

Method of test

In the present experiments the test fungi were cultured on a known volume (20 ml.) of filtered malt agar medium in a thin layer at the bottom of 500 ml. conical flasks stopped with the conventional cotton-wool plug. When the fungal colony had reached a width of about 3 cm., its mean diameter was measured from below. The toxic chemical was then introduced as described below. Subsequently, the flasks were further incubated at 22° C. and the size of the colony examined after 24, 48, 72 and 96 hr. If no further growth occurred after introduction of the chemical, transplants were taken from the edge of the colony and placed on fresh medium to determine if the fungus had been killed or merely inhibited.

The chemicals were (with the exception of water-soluble compounds) dissolved in a highly volatile solvent having itself negligible toxicity (usually petrol), and 1 ml. of the solution was added dropwise to 15 cm. diameter fluted filter-paper which absorbed this quantity entirely. The filter-paper was allowed to hang freely in the air for not more than a minute to allow the solvent to evaporate, and the folded filter-paper was then introduced into the flask, the plug of which was replaced by a well-fitting cork, having the surface in contact with the vapour covered with metallic foil (see Fig. 1). During the preparation and introduction of the filter-paper, some loss of the toxic substance under test might occur if it were highly volatile, but the volatility of compounds of practical interest is usually of an

entirely different order from that of the solvent, and losses of the toxicants can probably be disregarded as negligible when testing them in this way.

The test fungi employed were wood-rotting species that are generally used in the testing of wood preservatives in the laboratory.

Table 1 gives the results of tests carried out by this method. A geometrical series of concentrations was used, i.e. only 0·1, 1, 10 and 100 mg./500 ml. were normally included.

Fig. 1. Flask showing filter-paper in position over half-grown culture.

TABLE 1. *Summary of tests of toxicities of volatile antiseptics to wood-rotting fungi*

	Amount in mg. required to (I) inhibit, (K) kill the test fungi in 500 ml. flasks at 22° C.							
	Lentinus lepideus		*Fomes annosus*		*Polystictus versicolor*		*Coniophora cerebella*	
Substance	I	K	I	K	I	K	I	K
Dichlorphenoxymethyl chloride	10	100	10	100	10	100	10	100
Dichlorphenoxymethyl thiocyanate	1	10	1	10	10	100	1	100
Dichlorphenothiomethyl chloride	10	100	100	100	100	—	10	100
Xylene	100	100	100	100	100	100	100	100
Toluene		100		100		100		100
Para-formaldehyde	50	50	50	50	50	50	50	50
*Ortho*chlortoluene	100	100	100	100	100	100	100	100
Gamma-picoline	100	—	100	—	—	—	100	—
1-Methylnaphthalene	100	100	10	—	10	—	10	100
*Ortho*dichlorbenzene	10	100	10	100	10	100	10	100
Thymol		10	10	100	100	100		10
Para-nitrobenzyl chloride	10	—	10	—	100	—	10	—
Acetic acid	100		100				—	
Furfural	100	100	100	100	100	100	100	100

A dash indicates that the substance was not effective at 100 mg. concentration and a blank that it was not tested.

It will be noted that, of the substances tested, only the dichlorphenoxymethyl thiocyanate exceeded in effectiveness the well-known antiseptics *para*-formaldehyde and thymol.

Tests in wood

Blocks of spruce wood measuring $15 \times 5 \times 3{\cdot}5$ cm. were cut with the long axis at right angles to the grain of the wood, and a hole 1 cm. in diameter and 2 cm. deep was drilled in one face across the grain. The blocks were then autoclaved and infected with pure cultures of *Merulius lacrymans* or *Poria vaillantii*. After the fungi had grown through the blocks they were removed from the cultures, and 1 or 2 ml. of the substance under test were poured into the hole, which was immediately plugged with a sterilized wooden dowel. After 3 days small cores of wood were removed with a Pressler borer at distances of 2·5 and 5·0 cm. on each side from the edge of the central hole and placed in tubes of malt-agar medium and incubated to see if the fungus was still alive in them.

The results showed that the lateral penetration across the grain of the compounds tested was very poor, and that none was consistently effective at a distance of 2·5 cm. Good penetration of most of the fluids took place parallel to the grain along a path the width of the hole or slightly wider. This is in agreement with the results obtained by Vanine & Vladimarskaya, who measured the penetration of gases into wood by sealing wooden stoppers into glass vessels containing an indicator which reacted with the gas under test. They found that formaldehyde and carbon bisulphide penetrated wood in a radial direction to a depth of only 3 mm.

Practical applications

Results so far obtained suggest that fumigation of partially decayed wood with substances toxic to wood-rotting fungi is likely to present difficulties, since penetration of gases across the grain is slow. Barr, Thorogood & van Rest (1938) have shown that the rate of diffusion of hydrogen may vary between 0·005 ml./cm.^{2}/hr. per 1 cm. thickness, and that it may take 200 hr. for the concentration of hydrogen cyanide in the centre of 4×4 in. oak scantling to reach 7 mg./l., a quantity that would be toxic to insects such as *Lyctus* sp. In a later paper (Barr *et al.* 1940) showed that the amount of sorption of the gas (HCN) on the wood substance materially influences its rate of absorption.

It is obvious therefore that the rate of penetration and the amount of sorption of any chemical proposed for the fumigation of infected wood must be studied before a schedule of treatment with the particular substance can be proposed. The method described in this paper is intended for use as a screening test to be used for selection of volatile substances having an exceptionally high toxicity to fungi. Such materials will then have to undergo further tests to determine their physical properties and mammalian toxicity.

In addition to the direct treatment of wood infected with incipient decay there are other possible applications of fumigants against wood-rotting fungi, particularly in buildings infected with the dry-rot fungus, *Merulius lacrymans*. The mycelium of this fungus is sometimes found filling the cavity in hollow walls and in similar places inaccessible to other forms of treatment, and its successful control by use of formaldehyde vapour has been reported by Bayley Butler (1951). Again, it is sometimes desirable to kill spores of this fungus which may be widely distributed under a floor or on furniture, books and other objects which cannot conveniently be treated with a liquid antiseptic and where the use of a fumigant is clearly preferable. Results obtained by laboratory tests of the type described, on the toxicity to fungi of chemical substances, will be more directly applicable in cases where the mycelium or spores are freely exposed than when it is wished to treat wood in the solid where questions of penetration and absorption arise.

This work has been carried out as part of the programme of the Forest Products Research Board and is published by permission of the Department of Scientific and Industrial Research. The three phenoxymethyl compounds tested were kindly supplied by Messrs May and Baker Ltd., who state that the properties of these new compounds will be described in the chemical literature at a later date.

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PARTICLE SIZE OF INSECTICIDAL SUSPENSIONS AND THEIR CONTACT TOXICITY

IV. MECHANISMS OF ACTION OF DIFFERENT-SIZED PARTICLES

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Two earlier papers showed that in tests of suspensions by dipping, large crystals of DDT could kill grain beetles just as efficiently as colloidal DDT. But with rotenone, colloidal particles were far more toxic than large crystals; this difference was partly in their speeds of action. A third paper showed that in contact action the relative toxicity of small particles is increased by cooling the beetles after treatment. In tests by injection into milkweed bugs, particle size seemed to have no effect on toxicity of rotenone and DDT suspensions if the bugs were kept warm after treatment. In cool bugs, colloid acted more quickly than crystals, but the kills from the two types finally became the same. The time for this to come about was less for DDT than for rotenone and less still for DFDT, an analogue of DDT.

An explanation of these results is now given. In the action of contact poisons, attention is given to the waxy layer on the outside of the cuticle. Contact poisons must first of all dissolve in this wax layer, and it is suggested that the difference in action between rotenone and DDT is due to a difference in their solubilities in wax.

Small particles will always have the advantages, over large, of greater surface area and greater ability to enter openings in the body. With *Oryzaephilus surinamensis*, a main route of entry by rotenone into the body is possibly through the spiracles, and this may be why colloidal rotenone is so much more toxic than rotenone crystals. The solubility of rotenone in wax is thought to be small and if this is so, it will be easy to saturate the wax and the higher solubility of very small particles of rotenone will be of importance. The behaviour of rotenone particles of different sizes is therefore understandable.

Entry through the body openings is evidently unimportant for DDT, because large crystals kill as quickly as small ones. Penetration must be through the general cuticle. It is suggested that the solubility of DDT in wax is very high, compared with rotenone; the wax will not be easily saturated by DDT and small particles will not have the advantage of higher solubility, which is only helpful if saturation is reached.

It is shown theoretically that if solubility (in wax) does decide the relative behaviour of different-sized poison particles, then colloidal poison should be more toxic, relative to crystalline poison, if the insects are kept cool after treatment than if they are kept warm.

The following explanation is offered for the injection results. The blood of milkweed bugs contains free droplets of oil, and these are mainly responsible for carrying poison from injected crystals to the site of action. Colloidal poison probably diffuses there directly, and more quickly, in the aqueous phase of the blood. The difference in speeds of action of colloidal and crystalline poison will depend on the ratio of dose to solubility in oil. If this is large, saturation of the blood is easy, and

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small particles, with their special properties, act more quickly than large. If the ratio is small, the difference in speeds of action may not be detectable.

This seemed to hold for the three poisons tested, but not by any means exactly. The ratios for rotenone, DDT and DFDT were in the expected order.

Finally, it is suggested that in contact action, the relative speeds of kill of different-sized poison particles may be explained in a somewhat similar way, the solubility in cuticle wax, relative to the necessary dosage, being a controlling factor.

1. INTRODUCTION

Parts I, II and III of this series (McIntosh, 1947, 1949, 1951) described dipping, spraying, application and injection tests of simple aqueous suspensions of rotenone, DDT and the fluorine analogue of DDT, often called DFDT, against grain beetles and milkweed bugs. For convenience these papers are referred to as 'Part I', 'Part II' and 'Part III'.

From the dipping and spraying tests in Part I it is plain that DDT from large crystals (400μ) can easily penetrate the cuticle of grain beetles. Smaller particles are not so efficiently retained on the surface of the insects, but, once in position, are no more effective than large ones, weight for weight. Van den Hende & Jacobs (1950) have tested the types of suspension used in Part I as direct sprays and as films on glass and filter-paper against *Tribolium confusum*, with similar results.

On the other hand, Part II showed that toxicity of rotenone suspensions to grain beetles by dipping and spraying is inversely related to particle size, in spite of the low retention of small particles. The effects of time on kill were also measured; both colloidal and crystalline DDT gave kills which steadily increased as time went on. Crystalline rotenone behaved in the same way, but colloidal rotenone caused what seemed to be immediate paralysis, followed by recovery of some of the insects.

Part III showed that suspensions of different-sized DDT particles have different temperature coefficients of mortality. In consequence, the relative toxicity of small particles was increased by cooling the beetles after treatment. In injection tests on adult milkweed bugs, particle size seemed to have no effect on toxicity of rotenone and DDT suspensions if the insects were kept warm after treatment. But in cool insects, colloid acted very much faster than crystalline poison. However, the kills from the two types finally became equal. The time taken for this to come about was about 3 weeks for rotenone, 10 days for DDT, and less than 2 days for DFDT.

In the sections that follow, an explanation of these results is offered. §2B deals with Parts I and II and with the contact temperature-mortality tests of Part III; §2C with the injection tests of Part III.

2. EXPLANATION OF RESULTS

A. *Penetration of cuticle by poisons*

In dipping and spraying tests, a suspension of poison is applied to the insects; after this they dry, keeping back more or less poison on their bodies. The poison may

enter through openings in the body, or through the cuticle proper. Although the mouth-parts and spiracles may be closed during treatment they may open soon afterwards, admitting poison to the gut or tracheae. Both DDT and rotenone are efficient stomach poisons (Way, 1949; Shepard & Campbell, 1932), and it is known that derris dusts enter sheep keds chiefly through the spiracles (Webb, 1945). Whatever way penetration may take place, one might naturally expect that the smallest particles would be the most efficient, or would act the most quickly.

Rotenone conforms to this pattern; small particles are effective, and large crystals are very poorly toxic. However, insects can efficiently absorb DDT from large crystals as well as from small ones, and so an explanation of the results of Parts I and II will not be so much an explanation of the difference between the poisons, but of why DDT acts in this way.

To explain the results, it is necessary to give a short account of the nature of the cuticle and of a theory of drug action and its application to the present problem. A short review of recent papers on penetration is also given.

Structure of cuticle

A 'typical' cuticle has been described by Wigglesworth (1948). The structure of the epicuticle, or outermost group of layers, has received more attention than others, for it is the seat of the waterproofing mechanism of the cuticle. It is known to contain lipoids, both in the free form as waxes, and, in chemical combination with protein, as 'cuticulin'. The free waxes are chemically similar to beeswax, i.e. they are composed of long-chain esters, alcohols and fatty acids (Beament, 1948). A cuticulin layer forms the base of the epicuticle, and is covered by a polyphenol layer and a crystalline wax layer, which may itself be covered by a cement layer. These layers have different relative and absolute thicknesses in different species, or in different parts of the same species. A large number of pore canals cross the system at right angles to the surface, running inwards from the top of the cuticulin layer. Beneath the epicuticle lie the exocuticle and endocuticle, and the epidermal cells. The pore canals may reach to the base of the endocuticle or only to the outer endocuticle (Way, 1950). They may contain cytoplasm or, at a later stage, chitin (Dennell, 1943); or chitin and tanned protein (Way, 1950). Ducts from the glands in the epidermis also cross the whole cuticle, reaching to the cement layer, which is in fact secreted by dermal glands. The cuticle may bear bristles and sense organs.

It is assumed that the cuticles of adult *Tribolium castaneum* and *Oryzaephilus surinamensis*, the insects used in Parts I and II, are of this general type.

Recent work on penetration

It may be useful to summarize here a few of the recent papers on penetration of the cuticle by fat-soluble organic compounds acting as contact poisons. Some other

papers on this subject are quoted in more detail elsewhere in the text, and attention is called to the useful review by Metcalf (1948).

Obviously, poisons like DDT can enter the insect body easily; the cuticle hardly seems to be a barrier to them at all. Thus DDT, rotenone, γ-BHC, parathion and its analogue paraoxon are almost as toxic to adult American cockroaches by surface application as by injection (Tobias, Kollros & Savit, 1946; Dresden & Krijgsman, 1947; Metcalf & March, 1949).

Penetration may be equally fast through all parts of the cuticle, or faster in some spots than others. Entry of pyrethrins in oil occurs through the general cuticle of tick larvae and not preferentially through the spiracles, mouth or anus (Robinson, 1942). Toxicity of DDT has been found to be unrelated to general cuticle thickness in seventeen insect species (Bozkurt, 1948); to pulvillal cuticle thickness in houseflies, honeybees, milkweed bugs and German cockroaches (Sarkaria & Patton, 1949); and to tarsal and thoracic cuticle thickness in normal and DDT-resistant houseflies (March & Lewallen, 1950).

On the other hand, Bredenkamp (1942) found that some areas of isolated cuticle were more permeable than others to rotenone and other poisons. The structure of the tarsi or pulvilli of a species may decide how easily it is killed by DDT films (Burtt, 1945; Potts & Vanderplank, 1945; see also Hickin, 1945). The tarsi are the main points of entry of methylnaphthalenes, but not of rotenone, into third-instar larval Mexican bean beetles and adult pea aphids; the tarsi are not specially permeable to rotenone (Sun & Hansberry, 1947).

Hayes & Liu (1947) stated that adult houseflies, which have a thin tarsal cuticle, are more sensitive to DDT than some other species which have thicker tarsal cuticles (larval and adult Mexican bean beetles, and adult German cockroaches), but the relation was not proven. The progressive increase in resistance of growing tick larvae to pyrethrins has been attributed to progressive increase in thickness of their endocuticle (Robinson, 1942). In adult blowflies and German cockroaches, penetration of DDT or DDT in oil is said to occur only where the exo- and endocuticles are thin or absent, i.e. through the cuticles on joints or sense organs (Wiesmann, 1946, 1949).

Other workers have also shown that penetration takes place preferentially at well-defined points. The suggestion that it occurs mainly through the spiracles has been made by Roy, Ghosh & Chopra (1943), and by Roy & Ghosh (1944) using pyrethrum dusts on American cockroaches and the fly, *Chrysomyia megacephala*, and by Webb (1945) using derris dusts on mature sheep keds. Witt (1947), using larval, pupal and adult blowflies, concluded that DDT and γ-BHC enter through joints, sense organs and spiracles, and not through the general cuticle.

With *Rhodnius prolixus*, Wigglesworth (1942) showed that the entry of oils or pyrethrum in oil depends on the age and nutrition of the bug. In nymphs, penetration occurs most easily when the cuticle is stretched after feeding, for then the endocuticle is thin and entry may be through the pore canals which are short and

probably expanded. In older nymphs entry is more likely to be near the bristles; and in adults, by way of the dermal glands. Dennell (1943) also suggested that entry of insecticides may be along the pore canals (of larval fleshflies).

The above papers show that in contact action, penetration may be going on in one or more ways; these are not necessarily the same for different species-poison combinations. However, penetration of any solid poison must start with solution in the outermost layer of some form of cuticle.

As Wigglesworth (1948), Webb & Green (1945) and Webb (1949) have pointed out, the lower layers of the cuticle are permeated by water, which comes very close to the surface. It is useful to take Webb & Green's picture of the cuticle as a two-phase system as a greatly simplified model of cuticle structure. The thin wax and cement layers of the epicuticle form the external lipoid phase, covering an inner aqueous phase, representing the rest of the cuticle. On the basis of this, Webb & Green have been able to explain the synergism between poison dusts and various organic solvents, showing that the 'carrier efficiency' of a solvent depends on its physical properties. The solvent may help the poison to penetrate the wax phase; and if it has a high partition coefficient between water and beeswax and can, by its presence in the aqueous phase, increase the solubility of poison in water, then the rate of action of poison is greatly increased.

Physical drug action

At this point it will be helpful to outline a general theory of drug action, and return to penetration afterwards.

A distinction is made by Ferguson (1939) between poisons which act physically and those acting chemically. Physical action is found in cases of reversible narcosis, etc., and occurs when the poison molecules are large or chemically inert; chemical action is irreversible and involves poisons with small reactive molecules. Ferguson has analysed many cases of physical action, and introduced the idea that the best guide to toxicity is the chemical potential of the poison. The percentage of saturation of a poison in any medium is a measure of its chemical potential in the medium. If there is equilibrium among different phases containing a common solute, the percentage saturation in each phase is the same. Thus, if equilibrium exists, the chemical potential of the poison in the medium surrounding the animal is the same as at the site of action, which is nearly always unknown. The chemical potential is also the same in all other phases of the system, whether they act as insensitive intermediate phases, or take no part at all in bringing the poison to its site of action.

Different poisons show equitoxic effects at roughly the same percentage saturation in the applied medium; the value usually lies between 10 and 100 % of saturation. Ferguson believes that variations within this range are due to differences in chemical constitution, but they may also be due to differences in the rate at which equilibrium is reached. In a homologous series of organic compounds, the percentage saturation for equal effect gradually increases with the number of carbon atoms,

so that there is eventually a 'cut-off'. Above this critical point, homologues are poorly toxic, for the chemical potential cannot normally be greater than one (Badger, 1946).

Thus the characteristics of physical toxic action are (*a*) chemical inertness; (*b*) reversibility of action; (*c*) higher potency in a poorer solvent, and vice versa; (*d*) poison action at between 10 % saturation and saturation itself; and (*e*) a cut-off in homologous series.

(*a*). Although it is decomposed by air in the presence of light or alkalis (Fleck, 1948), rotenone is not specially reactive and can be absorbed by bean leaves and carried to other parts of the plant, apparently unchanged (Fulton & Mason, 1937). DDT is rapidly decomposed by bases, but is otherwise stable.

Martin & Wain (1944) have suggested that the toxicities of DDT and compounds of the same type depend on the ease with which they react in this way; but others have shown that this is probably wrong (Domenjoz, 1946; Müller, 1946; Browning, Fraser, Shapiro, Glickman & Dubrûle, 1948; Picard & Kearns, 1949; Cristol, 1950). It seems more likely that the two properties are not related, and that in the process of poisoning the DDT molecule remains intact.

(*b*). Welsh, Gordon, Prajmovsky & Easton (1945), and Welsh & Gordon (1947) have found that the action of DDT on crab nerves is reversible, especially in the presence of calcium ions. They suggest that it becomes adsorbed on the surface of the nerve sheath, its action being chiefly physical. Reversible action of DDT has been noticed in houseflies (Lindquist, Wilson, Schroeder & Madden, 1945), in adult mosquitoes (Kennedy, 1946) and in blowfly larvae (Hurst, 1949). Graph X of Part II shows that *Oryzaephilus surinamensis* can recover from poisoning by colloidal rotenone.

(*c*) and (*d*). Burtt (1945) found that both DDT and rotenone were more toxic to sheep ticks in poor than in good solvent oils; and in a review, Gavaudan & Poussel (1947) have calculated the percentage saturation of DDT at the toxic concentrations in water against several insect species, and found that it lay between 10 and 100 %.

(*e*). Testing homologous *n*-alkoxy analogues of DDT as space sprays against houseflies, and as mosquito larvicides, Prill, Hartzell & Arthur (1945) found maximum toxicity at C_2, i.e. at 1, 1-*bis*(*p*-ethoxyphenyl)-2, 2, 2-trichloroethane. The same peak of activity can be seen in the results of Lord (1948*b*) in tests of emulsions against chrysanthemum aphids and grain beetles.

Thus there is some evidence that both DDT and rotenone kill by physical processes. Lord (1948*b*) concluded that this was the case for DDT and its analogues. It is assumed that penetration itself is also a physical process, i.e. that neither rotenone nor DDT is chemically changed in passing through the cuticle (cf. Sharp, 1948).

B. *Penetration and particle size*

The first step in the penetration of the general cuticle by a pure crystalline poison in contact with the cuticle must be solution in the wax phase (wax or wax plus cement:

Webb & Green, 1945). This by itself is not enough to explain the toxic action; the poison must afterwards cross into the aqueous phase and be carried to its site of action by diffusion and by circulation of the blood. It is not clear how this comes about with pure poisons which are insoluble in water. Insecticides may be more soluble in insect blood than in water itself (Richards & Cutkomp, 1946); some further remarks about this are given in §2C.

If conditions are good, the percentage saturation of poison at the site of action will approach the percentage saturation in the wax phase, and will equal it if equilibrium is reached. The insect will die if there is a high enough concentration in the wax layer for long enough; the concentration needed to bring about death in a given time will vary from one individual insect to another, depending on their susceptibility. A certain amount of waste will always occur by detoxification and/or excretion.

The solubility of a solid in a solvent at any temperature depends on its particle size, and is inversely related to it (Jones & Partington, 1915). The particle size below which the increased solubility becomes noticeable depends on the solute-solvent pair, and for inorganic precipitates in water is commonly in the region of $0{\cdot}5\,\mu$ or less (Dundon, 1923). This property of small particles cannot be observed nor measured until saturation is reached.

An explanation of the particle size effects of rotenone and DDT can now be attempted on the basis of the above ideas. It is convenient to discuss the reaction of a single insect to a poison; but of course the only reliable measure one can get of this is some reaction of a group of insects, like percentage kill, median lethal concentration, etc.

Particle size and toxicity of rotenone (see Part II)

In Part II, the differences in potency amongst crystalline rotenone suspensions were relatively small, but colloidal rotenone was very much more toxic than any crystalline suspension. Thus in tests F, G and H of Part II, small hexagons ($20 \times 12\,\mu$) were only $3{\cdot}1$ times more toxic than large hexagons ($157 \times 118\,\mu$); but in tests Q and R, colloidal rotenone was 580 times more toxic than small hexagons.

These results might be explained if one supposed that the general body cuticle is not easily penetrated by rotenone, but that rotenone can enter the body through the mouth-parts into the gut, or through the spiracles. Colloidal particles would be able to enter body openings very much more easily than crystals. Rotenone can act as a stomach poison (Shepard & Campbell, 1932). But Webb (1945) showed that the chief route of entry of derris dusts into sheep keds is through the spiracles; penetration through the general cuticle is slight and at low temperatures negligible. For this reason one might suspect that it is entry through the spiracles, rather than the mouth-parts, that is the cause of the high potency of colloidal rotenone. The tracheal walls may either be more permeable to rotenone than the general cuticle, or form a short cut to the site of action.

The general cuticle of *O. surinamensis* must be slightly permeable to rotenone under the conditions used, because large crystals are slowly toxic; possibly a few small crystals can enter the spiracles from crystalline suspensions of rotenone, which are never absolutely uniform. Penetration probably takes place by both routes at the same time. With colloidal rotenone, penetration is chiefly through the tracheal cuticle; with crystalline rotenone, chiefly through the general cuticle. According to Wigglesworth (1947) and Richards & Korda (1948), the two types of cuticle have the same general structure, so the mechanism of penetration will be the same in each case.

Thus, the differences in potency amongst crystalline suspensions may be determined by the weight of rotenone they can deposit inside the tracheae; the coarser the suspension is, the smaller this will be. However, as a result of their special physical properties, small particles will have other advantages over large ones.

The solubility of rotenone in kerosene is very low (0·008–0·04 %; Jones & Love, 1937), and it is assumed that it is also sparingly soluble in cuticle wax. Because of their higher solubility and large surface area, small crystals will dissolve more quickly, giving a higher concentration of poison in the wax phase than large ones. In the case of colloid, the percentage saturation may exceed 100 %, i.e. the wax phase may in fact be supersaturated with poison; but even if this does not happen, and the spiracles do not form a short cut to the site of action, it is easy to see why colloidal rotenone should at least act more quickly than crystalline rotenone.

Evidently an insect retains, from a suspension of crystalline rotenone of the strength used in Part II, a dose of poison more than sufficient to kill it if the poison could only be absorbed efficiently. The rotenone content of the wax will increase to a steady value, which will depend on the rate at which rotenone crosses into the aqueous phase. The concentration will be maintained by the solution of more rotenone crystals, and the toxic effect will increase as time goes on.

With very small particles, a maximum concentration will be reached quickly, but not maintained from outside, because the insects do not retain much poison (Part II). Although the percentage saturation attained at first may be high, the total rotenone content of the wax phase will not be large. Burtt (1945) has emphasized that the percentage saturation of a poorly soluble substance can be seriously reduced by the removal of even a small amount of it. Thus, when rotenone diffuses into the aqueous layers, or becomes adsorbed on surfaces or dissolved in phases not concerned with the process of poisoning, the percentage saturation at the site of action may be reduced to a non-toxic level. Colloidal rotenone therefore causes paralysis at first, followed by recovery (Graph X, Part II).

These paragraphs do not explain why there is a real difference in toxicity between colloidal and crystalline rotenone in contact action, and not just a difference in speeds of action. If the site of action is reached by poison from colloidal rotenone by way of a short cut through the spiracles, then poison from crystalline rotenone,

travelling by a more circuitous route, may be more exposed to detoxification in the body. However, there is no evidence for this.

Particle size and toxicity of DDT (see Parts I and II)

DDT can be taken into the body just as easily from large crystals as from smaller ones (Part I); so penetration through body openings seems to be unimportant, although it may occur. The general cuticle must be easily penetrated, and it is necessary to assume that DDT is much more soluble than rotenone in wax, so that the wax layer is not easily saturated. DDT is known to be several hundred times more soluble than rotenone in kerosene, and is in general far more soluble than rotenone in natural oils (Jones & Love, 1937; Jones, Fluno & McCollough, 1945; Busvine, 1945; von Oettingen & Sharpless, 1946).

However, the wax layer is usually very thin (0·25 μ; Beament, 1945) and it would probably not be difficult to saturate, even though it may be able to dissolve much more DDT than rotenone. Possibly the cuticulin layer of the epicuticle also forms part of the 'wax phase'. Cholesterol is said to form an addition compound with DDT (Läuger, Pulver, Montigel, Wiesmann & Wild, 1945); if the wax contains sterols (Kühnelt, 1928) they may increase the solubility of DDT. The enhanced solubility of small particles only comes into play if a solvent is saturated with solute.

To produce equitoxic effects, crystalline and colloidal DDT suspensions must be applied at widely different concentrations, but the amounts retained by the insects are about equal (Part I). Smaller particles will give a more even distribution of poison. But if there is any difficulty in saturating the wax phase, the concentration produced in it by small particles may not be greater, by much or for long, than the concentration produced by the large crystals. The two forms will be equitoxic, on the basis of weight retained. If the colloid acts more quickly than the crystals, the difference in speeds of kill may have been missed in the tests of Part I; the insects were not examined until 2 days after treatment.

Some of the residual colloidal DDT may crystallize in the course of time, but that will not affect the general argument. DDT deposits are often reluctant to crystallize at all (Parkin & Green, 1947; Patton & Sarkaria, 1948; Busvine & Kennedy, 1949).

The solubility of DDT in wax being large, diffusion or adsorption will not reduce the percentage saturation so easily as in the case of rotenone, so that both types of DDT suspension will show an increase in kill as time passes.

Richards & Cutkomp (1946) have suggested that animals which have chitinous cuticles are more susceptible to DDT than those which have not, because the chitin concentrates the DDT from applied aqueous media (Fan, Cheng & Richards, 1948; Lord, 1948*a*). Chitin is not found in the epicuticle, but it is present in the exocuticle and endocuticle (Kühnelt, 1928; Wigglesworth, 1947). DDT may therefore become adsorbed on the surface of the chitin crystallites in these regions (Fraenkel & Rudall, 1940). Whether chitin plays any part in the entry or poison

action of DDT or not, the adsorbed layer can be treated like a surface phase, as Ferguson suggested (1939). If there is equilibrium, the percentage saturation in each phase will be the same, and the above arguments will still apply.

Effect of temperature on contact toxicity of DDT suspensions (*see Part III*)

It has been said that the first step in penetration of the cuticle must be solution of poison in the wax phase. The solubility of poison in wax will be affected by temperature and will presumably increase if temperature is raised.

The solubility (S_r) of very small particles of radius r is expressed by

$$\frac{RT}{M}\log\frac{S_r}{S_\infty}=\frac{2\sigma}{\rho r}$$

(Jones & Partington, 1915). Here R = gas constant; T = temperature; M = molecular weight of solute; S_∞ = normal solubility of solute; σ = solute-solvent interfacial tension; ρ = density of solute.

R and M will be constant in any one system, and if variation in temperature is not great, ρ and r may also be taken as constant.

Let $2M/(\rho rR)=k$, a constant. Comparing two temperatures, T_1 and T_2 ($T_2>T_1$),

$$\text{at } T_1\text{:}\quad \log\frac{S_{(1)r}}{S_{(1)\infty}}=\frac{k\sigma_1}{T_1},$$

$$\text{and at } T_2\text{:}\quad \log\frac{S_{(2)r}}{S_{(2)\infty}}=\frac{k\sigma_2}{T_2}.$$

As σ usually decreases as temperature increases,

$$\frac{\sigma_1}{T_1}>\frac{\sigma_2}{T_2},$$

and so

$$\frac{S_{(1)r}}{S_{(1)\infty}}>\frac{S_{(2)r}}{S_{(2)\infty}}.$$

Consequently, if the temperature is lowered, the ratio of solubilities of small: large particles will increase. The solubilities of both will decrease, but by different amounts.

One might therefore expect that if the insects were kept cool after treatment, colloidal poison would be more toxic, relative to crystalline poison, than if they were kept warm. This should be a general property of poisons; the results of Part III showed that it is found with DDT. The relative killing powers of DDT suspensions clearly depended on temperature, and changed in the direction one might expect if the explanation outlined above is correct. No explanation is offered for the negative temperature coefficient of mortality shown by DDT.

C. *Transport of poison by blood after injection* (*see Part III*)

If a poison is injected into the body cavity of an insect, it will come into contact with the blood at once and will diffuse through or be carried by it to the site of

action. The solubilities of rotenone and DDT in water are extremely low (Jones & Love, 1937; Richards & Cutkomp, 1946), and if the blood were simply an aqueous solution, there would be no reason to believe that their solubilities in it differed by much. They would both be low, and one might expect that in both cases the smallest particles would be the most toxic, or would act the most quickly. But milkweed bugs have free fat droplets ('lipomicrons') in their blood, which can be thought of as a dilute aqueous emulsion of oil. The oils from whole pupae of silkworms and of tent moths are rather similar to olive oil (Bergmann, 1936), but nothing is known about the oil in the blood of milkweed bugs.

An injected poison will dissolve in the aqueous and oil phases of the blood, according to its solubility and distribution coefficient. With both crystalline rotenone and crystalline DDT, most of the poison will dissolve in the oil phase. By diffusion and circulation, the poison will be taken to its site of action. It is unlikely that crystals will be carried there bodily by the blood; they will more likely become lodged in the body cavity and dissolve in the blood. Colloidal poison will probably be able to diffuse to the site of action directly, and therefore more rapidly, without first going into solution in the oil.

The speed at which transport to the site of action goes on will depend on the solubility and rate of solution of the poison, and on the rate of diffusion and possibly of heart-beat. All of these are determined by temperature. Rate of solution will of course be inversely related to particle size.

It will be necessary to discuss the results of the injection tests of Part III one by one.

Tests on warm insects

If the milkweed bugs were kept at 27° C., the kills from crystalline and colloidal suspensions of rotenone were equal after 2 days. This is taken to mean that in 2 days all the crystalline rotenone is in solution; it may be in the aqueous phase of the blood, the lipomicrons, the fat-body, the site of action or elsewhere in the body. But if it had not all dissolved, one would expect the colloid to be the more toxic.

Crystalline and colloidal suspensions of DDT were also equitoxic after 2 days. If the final median lethal concentration (M.L.C.) figures for colloidal suspensions are taken as end-point M.L.C. values for the two poisons (Beard, 1949), then the end-point M.L.C. for DDT is about 38 times more than for rotenone (see Table 3 of Part III* and Table 1 of this paper). However, DDT is more soluble than rotenone in olive oil by a factor of about 50 (Table 1); the injected DDT crystals all dissolve within 2 days.

Tests on cool insects

When insects were injected with rotenone suspensions and kept at 10° C. after treatment, the colloid seemed to be very much more toxic than the crystalline

* In the first column of Table 3, Part III, the letters H, I, J have unfortunately been omitted.

suspension after 2 days. Afterwards, as time passed, the kill from the colloid increased very slowly indeed, but the kill from crystalline rotenone increased throughout the test, quickly at first, and then more slowly. After about 3 weeks the kills from the two types become roughly equal.

At 10° C., circulation and diffusion are slower than at 27° C., so the whole process of dying takes longer; in addition, saturation of the blood by poison is more easily attained, and the special properties of small particles are of importance.

Because of their greater surface area and solubility, small particles dissolve more quickly and give a higher concentration in the blood than large particles do; in 2 days, a maximum concentration at the site of action has been reached or very closely approached. Rotenone from large particles, which dissolve more slowly and are less soluble, arrives at the site of action more slowly, so that after 2 days the colloidal suspension seems to be the more toxic.

TABLE 1. *Solubilities of poisons in olive oil; and their end-point* M.L.C. *values obtained in injection tests of colloidal suspensions against adult milkweed bugs* (Oncopeltus fasciatus)

Test in Part III	Temp. of test (° C.)	Poison	Solubility in olive oil (g./100 ml.)	End-point log (M.L.C. × 10^4) for colloid
E	27	Rotenone	0·2*	1·20
F	27	Rotenone	0·2*	1·20
G	27	DDT	10*	2·80
H	27	DDT	10*	2·74
I	10	Rotenone	0·004	0·97
J	10	Rotenone	0·004	1·06
K	10	DDT	6·5	1·93
L	10	DDT	6·5	1·78
M	10	DFDT	47	2·66
N	10	DFDT	47	2·47

* Jones & Love (1937), Busvine (1945), von Oettingen & Sharpless (1946).

After this, the kill from the colloidal rotenone increases only very slowly, probably because the more resistant insects die more slowly than the susceptible ones. But in insects treated with crystalline rotenone, only a fraction of the poison has arrived at the site of action in 2 days. More of it continues to arrive from the dissolving crystals as time goes on; the kill increases at a measurable rate until finally the kills from the two types of suspension are equal, the crystals having completely dissolved.

Thus, the difference between the rotenone suspensions is in their rates of kill, and it is suggested that this is because of the difference in surface area and solubility between large and small particles.

If one used an equally potent but more soluble poison, saturation would be less easily reached; and if the poison were so soluble that the blood were never saturated, then it would not take very long for the kills produced by large and small

particles to become equal. The difference in speeds of action will be low if it is not easy to saturate the blood.

The solubilities of poisons in olive oil at 10° C. have been found by adding successive small amounts of solid to measured volumes of oil, until no more dissolved. The figures for rotenone, DDT and the fluorine analogue of DDT (DFDT) are shown in Table 1.

When colloidal DDT and short needles were compared against milkweed bugs at 10° C., the colloid was more toxic than the crystals by about 15 times after 2 days. The kills became the same after about 10 days.

DDT is so much more soluble in oil than rotenone that saturation of the blood is less easily attained, even though a higher dose is necessary (Table 1); the ratio of end-point M.L.C. to solubility in olive oil at 10° C. is a great deal smaller for DDT, which shows a smaller difference than rotenone between the speeds of action of large and small particles.

Crystalline and colloidal suspensions of DFDT were equally toxic in 2 days at 10° C. Again, the ratio of end-point M.L.C. to oil solubility at 10° C. is a measure of the ease of saturation of the blood. It is slightly smaller for DFDT than for DDT (Table 1), but needles of DFDT cause maximum toxicity much more quickly than needles of DDT. This suggests that there is a difference between their speeds of solution in the oil phase of the blood. However, no difference could be found between the rates of solution of powdered DDT and DFDT in olive oil; both reached 50 % saturation in 48 hr. at 10° C. It may be that crystalline DFDT is soluble enough in the aqueous phase of the blood for transport in it to be of importance. So far it has been assumed that the crystalline poisons dissolve only in the oil phase. This point has not been cleared up.

With all the poisons used in the injection tests the particle size range was about the same; colloidal particles were compared with crystals of about 50μ. It is suggested that the effect of particle size on speed of killing by injection may in some circumstances depend on the ratio of end-point M.L.C. to solubility in oil. If this is so, and the ratio is large, saturation is easily achieved and small particles act more quickly than large ones, weight for weight, and may therefore seem to be the more toxic. When the ratio is small, saturation is more difficult, and the difference in speeds of action may not be noticeable. If the ratio varies with temperature, it may determine how the difference in speeds of action varies with temperature.

Finally, it is suggested that the relative speeds of killing of different-sized poison particles by contact action may be explained in a somewhat similar way, the solubility in cuticle wax, relative to the necessary dosage, being a controlling factor.

3. Discussion

The explanations of toxic action in this paper greatly over-simplify the picture; they are really only suggestions on which it is intended that future work may be based.

The results of the tests on temperature coefficients of DDT suspensions by dipping might be explained, not as suggested in §2B, but in the following way. Although deposits of DDT are normally slow to crystallize, it may be that the deposit from colloidal DDT crystallizes more quickly on the insects at 25 or 30° C. than at 12° C. If so, the deposit obtained at 25° C. might soon become completely crystalline, and not very different from the one left by needle aggregates; the two deposits would be equitoxic, weight for weight. At 12° C. the small particles, with their physical advantages, would be more toxic. However, the results of the injection tests at 27 and 10° C. make this explanation seem less likely.

Penetration may not take place by diffusion through the bulk of the cuticle, but by two-dimensional diffusion along fat/water interfaces in the cuticle, as proposed for fatty acids or pyrethrins (Hurst, 1943). DDT may be carried through the body by way of the nerves (Läuger, Martin & Müller, 1944; Heubner, 1949). These facts need not affect the general argument, for the first step towards poisoning after application or injection of a poison must be its solution in some lipoid phase.

In the discussions in §2, little account has been taken of detoxification or excretion of poison, processes which may be going on at the same time as those leading to death. Ferguson & Kearns (1949) have shown that when DDT is injected into milkweed bugs in acetone solution, which will probably produce a colloidal suspension inside the body, about 90 % of the poison is chemically changed to an unknown product within 90 min. The temperature was not given. The fact that, in tests G and H of Part III, needles of DDT are as toxic as colloidal DDT after 2 or 3 days at 27° C. suggests that DDT from both suspensions follows the same route to the site of action, and that in 2 days the DDT from each of the suspensions has been decomposed to the same extent. If this were not so, it would be hard to understand how the two suspensions could finally give the same kill.

Hurst (1949) has explained the negative temperature coefficient of mortality of DDT by saying that the temperature coefficient of poisoning is smaller than the temperature coefficient of detoxification. At a lower temperature DDT is in fact more stable in the insects relative to its rate of killing; this may explain how the kill of DDT needles at 10° C. can continue to increase even 10 days after treatment (tests K and L of Part III). Colloidal DDT reaches the site of action rapidly, but is decomposed to the same extent.

In my tests the relative potencies were clearly dependent on temperature. The results of this series re-emphasize what has been said by others (e.g. Potter & Gillham, 1946), namely, that the figures one obtains when comparing different poisons depend on the insect species and particle sizes used, on the method and temperature of the test, and on the time between treatment and inspection of the insects. This applies to both position and slope of probit line. A difference in 'kill' measured in some standard way may mean that there is a real difference in toxicity. But one cannot be sure of this; the difference may just be in speed of killing, in which case it will grow smaller or even disappear if the insects are kept

and reinspected often enough (Beard, 1949). In tests of rotenone by dipping (Part II) and by application, the differences between colloidal and crystalline suspensions persisted; they became a little smaller during the test, but never disappeared. But in all the injection tests any differences were only in speed of action.

The ratio of end-point M.L.C. to solubility in olive oil, besides possibly being a guide to the difference in speeds of kill of large and small particles, is also a measure of the percentage saturation of poison in body fat at the M.L.C. level. Although the total weight of fat in a bug is unknown, the two values will be proportional to one another. According to Ferguson (1939) the ratios for the three poisons should have roughly the same value if they act physically; but it is easily seen from Table 1 that this is not the case. However, the end-point potencies of the three poisons are arranged in the order that Ferguson's theory predicts. Their potencies are not proportional to the molar concentration of poison in body fat, as one would expect from the Overton-Meyer theory of narcosis.

The number of lipomicrons in the blood of milkweed bugs does not seem to change even after a week's starvation; and the bugs will not readily take other food in place of milkweed seeds. It would be interesting to do injection tests on insects which have no lipomicrons, or on cockroaches, in which the number of lipomicrons depends on the diet and on the time since feeding (Haber, 1926). If the fat content of the blood were low, one might expect that the difference in speed of action between colloidal and crystalline poisons would be accentuated, and vice versa.

Throughout the series only three poisons have been used. Future tests will apply the same methods to other poisons which can be obtained as pure crystals, including, for example, the dibromo analogue of DDT, which is less soluble, and aldrin, which is more soluble than DDT. Like DFDT, aldrin is rather volatile, and would have to be tested by injection. The results might make it possible to decide whether solubility in wax or oil, and rate of solution, are really properties which determine the relative toxicities or speeds of action of large and small particles of poison.

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PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Ordinary Meeting of the Association held on Wednesday, 18 April 1951, in the Imperial College of Science and Technology, London; the President, Mr R. W. Marsh, in the Chair.

The following papers were read and discussed:

1. The action of metaldehyde on slugs. By Prof. J. B. Cragg and Mrs M. Vincent.
2. Problems in the bio-assay of potato root diffusate. By Mr D. W. Fenwick.
3. Stored-products insect control in the tropics of south-east Asia, the Middle East and Central America. By Mr R. H. Davis.
4. The chemical control of the apple sawfly. By Dr A. H. M. Kirby.
5. Effects of time of sowing and singling sugar beet on yellows virus infection. By Mr J. W. Blencowe.
6. Wind tunnel experiments on particle deposition. By Dr P. H. Gregory.

Visit of the Association to the Butterwick Research Laboratories, 1 June 1951. Under the general direction of Dr P. W. Brian, the following topics were demonstrated: Antibiotic production by soil fungi; Alternaric acid -a phytotoxic substance produced by *Alternaria solani*; Griseofulvin—*in vitro* activity and use as a systemic fungicide; Nitrogen metabolism of fungi; Toxicology of insecticides; Exfoliative cytology; Organic chemistry of griseofulvin and gladiolic acid; Infra-red studies on viridin; Fungicidal activity in relation to chemical structure.

THE CHEMICAL CONTROL OF THE APPLE SAWFLY

By A. H. M. Kirby, *Research Station, East Malling, Kent*

Although this pest, *Hoplocampa testudinea* (Klug), was described by Prof. J. O. Westwood as far back as 1847, and by Miss Ormerod in 1898 and Prof. Theobald in 1909, no hope of chemical control seems to have been discovered until 1924, when Grubb & Bagenal reported the results of fungicide trials at East Malling on Worcester Pearmain, which is the variety most frequently attacked. These workers tried adding lead arsenate to pre- and post-blossom fungicide sprays; pre-blossom arsenate gave no control of sawfly, but the reduction in attack following arsenate application 'immediately after the blossom fell' was of the order of 70–80 %, in the presence of 1–3 % lime-sulphur. Petherbridge & Tunnington (1929) failed to achieve control with lead arsenate alone in 1926, but turned to nicotine with success in the following year. In 1928 they reduced an infestation of 85–90 % to 30 % with one spray of nicotine (7 days after petal-fall), and to 5 % where the first spray was followed by a second at the same strength, 0·0625 %, 7 days later; two sprays gave them more apples than did one, but the fruit size was less.

By 1935, work at Long Ashton and East Malling showed nicotine plus a wetter to be superior to lead arsenate, and the latter was dropped. Derris was tried as an alternative,

and was recommended by Kearns & Martin (1938) for use, with oil, against plum sawfly. But it proved inferior to nicotine for apple sawfly control, except for control of the migrating larvae which is only of secondary importance.

Until recently, nicotine has been the accepted chemical for control of apple sawfly. Timing of the application to coincide with egg-hatch has been regarded as important, but Hey & Steer (1934) obtained excellent control on several varieties of apple by applications up to 6 days prior to egg-hatch, and it has been customary for growers to apply nicotine for apple sawfly control in the first post-blossom fungicide spray. This is usually applied at 80 % petal-fall, and it is curious that the Ministry of Agriculture Advisory Leaflet on apple sawfly advises growers, when applying two sprays, to put the first on at 80 % petal-fall and the second a week later, keeping the fungicide for the *second* spray. Nevertheless, nicotine has given erratic results, and a more reliable spray-material is called for. With this end in view, Davies & Eaton (1950) began trials in 1946 with DDT and two Lethanes; DDT, at 0·05 % with Triton X 100 as wetter, gave no control when applied at petal-fall and poor control at egg-hatch, and the two Lethanes were no better. In 1947, HETP was tried at petal-fall and at egg-hatch but failed to give any control; this result fits in with the view that a stable insecticide is required, even the period of egg-hatch exceeding the persistence of HETP. γ-Benzene hexachloride, on the other hand, gave a high degree of control whether applied at 80 % petal-fall or at peak egg-hatch. This result was confirmed in 1948, when Davies & Eaton also obtained excellent results with parathion and chlordane. It was therefore clear by that time that at least three modern insecticides having greater persistence than is usually attributed to nicotine were very promising for apple sawfly control.

Further studies were, however, necessary for several reasons, and the work in 1949 and 1950 was undertaken* with the following points in mind:

(*a*) consistency in performance from season to season;
(*b*) the performance of further new insecticides;
(*c*) the mode of action of the insecticides employed;
(*d*) weather and other factors involved in the success of chemical control measures.

The 1950 trial was carried out in the orchard used by Davies & Eaton during 1946–8, using trees of the same variety, Worcester Pearmain. The 1949 trials, however, had to be made elsewhere, and large commercial trees in an adjacent orchard, Worcester Pearmain and Cox's Orange Pippin, were used. This necessitated a revision of our programme, and only one new material, namely, parathion, was compared with the standard spray of nicotine. Treatments consisted of parathion at 0·02, 0·005 and 0·00125 % respectively, emulsified with 0·02 % MB320, 0·2 % diacetone alcohol and 0·0125 % Aerosol OT; nicotine as standard was used at 0·05 %. The first three sprays were applied rather before 80 % petal-fall on randomized blocks with four replicates; the lowest concentration was also applied at egg-hatch on a single block. Nicotine was applied by the farm on the rest of the orchard and records were taken on a single block of these trees; our unsprayed controls consisted of a single block in the middle of the parathion replicates. Nicotine was a complete failure, but parathion at 0·02 % gave a 95 % reduction of a 50 % attack on Worcester Pearmain; reduction was significantly less as the concentration of parathion was lowered. The egg-hatch spray of parathion was rather more effective than the same strength applied at petal-fall.

In 1950, we returned to the small Worcester Pearmain trees at the Station and a range of insecticides was employed. DDT was brought back at a higher concentration, γ-BHC, chlordane and parathion were tested at two concentrations each, and toxaphene, aldrin and dieldrin were brought in for the first time. γ-BHC was also tested as a mixture with 1 % lime-sulphur. All materials, including nicotine, gave over 90 % reduction in an infestation of over 40 %, which is ample commercial control, and γ-BHC proved quite as active in the presence of 1 % lime-sulphur as without it. The first six treatments were significantly better than nicotine, and parathion and γ-BHC can both be said to have been consistently effective

* In collaboration with Mr K. S. McKinlay and Mr R. G. Gambrill.

for 3 years. Aldrin and dieldrin are obviously very promising, with toxaphene close behind, but chlordane, which was again effective, offers no obvious advantages and is not available in this country.

Turning to the problem of mode of action, that is, to the question of whether any of these materials were ovicidal or not, Mr McKinlay made careful records in both years of the progress of the eggs and larvae. Kearns (1933) had expressed the view that nicotine could kill the eggs in the later stages of development, and Hey & Steer (1934) interpreted their results to mean that nicotine is ovicidal. There is nothing in the evidence of these latter workers to preclude the view that the larvae died after hatching, and this view was later taken by Kearns, Marsh & Martin (1936), who found all eggs to hatch but the larvae to die subsequently, presumably as a result of eating some of the calyx tissue. This implies the retention of the nicotine as a salt of one or more organic acids in the calyx tissue, and a stomach-poison mechanism of action for nicotine in the case of this pest. Mr McKinlay found no evidence in either year that the larvae were affected prior to eclosion by any of the chemicals used (McKinlay, 1950). In 1949, the larvae on nicotine-sprayed trees, and often on the trees receiving the lowest concentration of parathion, not only hatched but entered the fruitlets and burrowed normally; larvae on fruitlets sprayed with 0·02 % parathion were found dead or moribund near to or even within remnants of the egg-shell. Similarly, in 1950, eggs developed and hatched normally regardless of chemical treatment, and differences between effects of treatments showed up after hatch. The percentage of larvae alive or dead within 24 hr. of hatching following various spray treatments showed that γ-BHC and parathion killed larvae very soon after emergence, but that all larvae on fruitlets sprayed with nicotine were alive for at least up to 24 hr. The other insecticides showed an intermediate speed of action, toxaphene being rather slow although quite effective.

The fourth aspect of this problem which we had in mind was the effect of meteorological and biological factors on the efficiency of our sprays. The variation in the efficiency of nicotine against this pest which led to our work has been ascribed to weather conditions at the time of spraying. Thus Kearns, Marsh & Pearce (1933) called for relatively high temperatures for spraying and regarded 55 F. as minimal for any reasonable degree of control. This view was admissible so long as nicotine was believed to act as a contact agent on the newly emerged larvae, but may well be expected to be untenable for sprays applied a week before egg-hatch. The failure of nicotine on the farm where we were working in 1949 was, it is true, associated with spraying at about 56° F., but the excellent results in 1950 followed spraying at 55° F. The variation from 96 to 44 % control experienced by Davies & Eaton in the years 1946–8 was also associated with almost identical spraying-day temperatures in those 3 years. Thus it appears that the temperature on the day of application is not important. But in 1946, when Davies & Eaton obtained so little control with nicotine, there was an unusually prolonged period between spraying and egg-hatch. This suggests that the real disadvantage lies in cold weather subsequent to spraying; good results may follow spraying on a cold day, provided the weather warms up soon afterwards. These remarks do not apply to control of aphids and other insects when nicotine acts as a contact insecticide, but only to apple sawfly.

The action of rain does not seem to be important. In 3 days after spraying much more rain fell in 1947 and in 1950 than in 1946, but control was superior in those 2 years. There was no measurable rain in 1949 for 5 days after spraying compared with half an inch in the same period in 1950, and we have concluded that: (*a*) nicotine does not remain in a form liable to leaching by rain, and (*b*) that the failure of nicotine in 1949 was due at least as much to inefficient spraying as to any other cause.

Kearns *et al.* (1935) reported that the attack in their 1934 trial was mainly on blossoms which failed to set. This was also our experience in 1950, when the early blossoms set but were not stung. In that Long Ashton trial, instead of total loss of crop as would normally follow their degree of early infestation, only a small increase had occurred 1 month later. There is therefore reason to suppose that the effect of our sprays in 1950 would have been

even more spectacular in a more normal year. At least three factors seem to be involved in this question of infestation: (*a*) the period at which the variety blossoms, (*b*) the period during which the sawflies lay their eggs, and (*c*) the period during which pollination takes place. If (*c*) is late relative to (*a*), early egg-laying will fail to give much infestation, whereas late-laying may lead to total loss of whatever crop there is. Unless adequate unsprayed controls are included, a so-called trial could lead to quite spurious results in a year when the initial infestation was unable to develop.

A few other considerations remain to be mentioned. In our experience, only parathion, of all the compounds tested for apple sawfly control, has caused any sign of phytotoxicity. In 1949, there was up to 5 % early yellowing and fall of primary leaves on Cox trees, proportionate to the concentration of parathion. Neither parathion nor any of the other compounds caused any damage on Cox trees in 1950. No damage followed the use of γ-BHC and lime-sulphur on Worcester Pearmain in 1950, and applying parathion with lime-sulphur in 1949 did not seem to diminish its value for sawfly control. Toxaphene appears to be compatible with lime-sulphur, and aldrin and dieldrin are certainly alkali-stable.

Comparatively little is known about the effect of these insecticides on predatory and parasitic fauna of the orchard, and our trials afford no indication. We understand from Dr Collyer that both parathion and γ-BHC, at 0·01 %, are toxic to the most important predator of the fruit tree red spider mite in Essex for at least a fortnight after spraying, and there is reason to suppose that a build-up of the mite may follow applications of these insecticides late in May.

However, quite good evidence is now available in the literature that γ-BHC and parathion are highly toxic to honeybees and, presumably, other pollinating insects visiting sprayed blossom. Toxaphene is clearly much less toxic to honeybees, and this is a strong recommendation for a material designed for use at 80 % petal-fall. No data are available for dieldrin, but aldrin seems to behave more like toxaphene than the others as a contact insecticide (Eckert, 1949); more data are required.

A tentative recommendation would be to spray BHC 1 week after 80 % petal-fall, but it must be remembered that the later the spray is put on, the more danger there is of a deleterious effect on red spider predators. Also as Miles (1932) suggested, closure of the calyx-cup after petal-fall may prevent the entry of the spray into the cup, especially in the variety which is most susceptible to sawfly attack, namely, Worcester Pearmain. There is, I think, still reason to search further for a safe chemical to apply at 80 % petal-fall which is the latest at which the fungicide spray should be applied; toxaphene may prove to be the suitable material, and this is now being made in this country, but aldrin has claims also and may be available before long.

It is hoped to continue the search for an efficient insecticide for apple sawfly control, which will be sufficiently specific to leave the predators of the fruit tree red spider mite unharmed, but progress will inevitably be slow as neither apple sawfly nor the predators can be subjected to laboratory screening.

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CONVERSAZIONE

A Conversazione on recent advances in applied biology, organized by Dr Marion Watson, was held on 27 July 1951 in the Botany Department of the Imperial College of Science and Technology (by kind permission of Prof. W. Brown).

A classified list of the exhibits is given below.

Virus diseases of plants and insects

A.R.C. Plant Virus Research Unit, Molteno Institute, Cambridge

The polyhedral virus diseases of insects. Kenneth M. Smith and N. Xeros

East Malling Research Station

A virus disease of gooseberries and some virus diseases of cherries. A. F. Posnette

Rothamsted Experimental Station, Harpenden

The effect of high temperatures on the establishment and multiplication of some plant viruses. B. Kassanis

The use of radioactive phosphorus to study aphid feeding. H. Nixon and Marion Watson

Rothamsted Field Station, Dunholme, Lincs

The sugar-beet yellows problem in the field. R. Hull, C. E. Cornford and L. F. Gates

General and agricultural mycology

A.R.C. Potato Storage Investigation, Univ. of Nottingham School of Agriculture

Mutations appearing in *Fusarium caeruleum* cultures treated with tetrachlornitrobenzene. R. K. McKee

Crawley Research Station, Sussex

The influence of chlorinated nitrobenzene compounds on fungal growth. E. D. W. Wiggins

East Malling Research Station

Physiological studies on verticillium wilt of hops. P. W. Talboys

Rothamsted Experimental Station, Harpenden

Fungi causing eyespot lesions on cereals (*Cercosporella herpetrichioides* Fron., *Corticium* (*Rhizoctonia*) *solani*, *Gibellina cerealis* Pass.). Mary D. Glynne

Royal College of Science, South Kensington

Some methods of measuring the susceptibility of potatoes to fungal attack. P. K. Isaac

Nematode diseases of plants

East Malling Research Station

The etiology of strawberry cauliflower disease. R. S. Pitcher and J. E. Crosse

East Malling Research Station and Rothamsted Experimental Station

The nematode diseases of strawberry caused by *Aphelenchoides fragariae*, *A. ritzema-bosi* and *Ditylenchus dipsaci*. R. S. Pitcher, Mary T. Franklin and J. B. Goodey

Rothamsted Experimental Station, Harpenden

Some hosts of *Ditylenchus destructor*, the potato tuber nematode. J. B. Goodey

A new species of *Aphelenchoides* attacking *Scabiosa caucasica*. Mary T. Franklin

General and applied entomology

D.S.I.R. Pest Infestation Laboratory, Slough

The insect fauna of old birds' nests with special reference to household pests. G. E. Woodroffe

The treatment of small individual insects with single drops of insecticide by means of a micro-drop applicator. P. S. Hewlett

Imperial College Field Station, Sunninghill, Berks

Illustrations of the structure of the tarsi of species of blowfly and tsetse fly. C. T. Lewis

Ministry of Agriculture Infestation Control Division, Tolworth, Surbiton

Control of insect pests in stored products. J. A. Freeman

N.A.A.S. (W. Midland Province), Woodthorne, Wolverhampton

Soil pests: carrot fly control: the winter moth complex. H. C. F. Newton

Rothamsted Experimental Station, Harpenden

Model illustrating the relation of numbers of insects trapped to maximum and minimum temperatures. C. B. Williams

Aerial density and fluctuations of *Aphis fabae* in relation to temperature and wind speed. C. G. Johnson

Agricultural zoology

Ministry of Agriculture Infestation Control Division, Tolworth, Surbiton

Control of rabbits: work on *Glis glis*, the edible dormouse. J. A. Freeman

N.A.A.S. (Eastern Province), Anstey Hall, Trumpington, Cambridge

Damage caused by rabbit grazing in winter corn. H. C. Gough

N.A.A.S. (W. Midland Province), Woodthorne, Wolverhampton

Rabbit damage to cereals, fruit trees and rhubarb. H. C. F. Newton

Plant nutrition

Cambridge University Botany School

The development of mycorrhizal roots of *Pinus sylvestris*. N. Robertson

Long Ashton Research Station, Bristol

The use of *Aspergillus niger* for determining copper, zinc, manganese and molybdenum in soils. D. J. D. Nicholas

Zinc and copper deficiencies in apple and pear. C. Bould

Royal College of Science, South Kensington

Photosynthesis of starch by tobacco with ^{14}C-labelled carbon dioxide. Helen K. Porter and R. V. Martin

Weeds, weedkillers and antibiotics

Ministry of Agriculture Plant Pathology Laboratory, Harpenden and N.A.A.S. (Eastern Province), Anstey Hall, Trumpington, Cambridge

Ear distortions on cereals caused by spraying with MCPA and 2:4-D. E. C. Large and W. A. R. Dillon Weston

Rothamsted Experimental Station, Harpenden

Species and varieties of wild oats occurring in the British Isles. Joan Thurston

Royal College of Science, South Kensington

The production of antibiotic substances in organic manures. Erna Grossbard

Instruments and apparatus for use in biological research were exhibited by: Messrs C. Baker of Holborn; Baird & Tatlock (London); Cooke, Troughton & Simms; E. M. Cromwell & Co.; Dawe Instruments; Labgear; L. Oertling; Sartory Instruments; Stanton Instruments; Techne (Cambridge); W. Watson & Sons.

REVIEWS

Introduced Mammals of New Zealand: an Ecological and Economic Survey. By K. A. Wodzicki. Bull. 98, N.Z. Dep. Sci. Industr. Res. 1950. 12*s.* 6*d.*

As is well known, geographical isolation allowed the development in New Zealand of a remarkable fauna. With the exception of the native dog and rat, introduced about the fourteenth century, there was a complete absence of placental mammals until the introduction of domestic animals and the black rat, by Captain Cook in 1774, and the brown rat and house-mouse soon afterwards.

The early settlers in New Zealand found a vertebrate fauna mainly of birds and fish and introduced, with official approval, many species of mammals and birds, mostly from England. The animals were imported in the first place for food, but also to remind the pioneers of home and, later, for sport and the profit of their skins.

Some species did not survive but many, finding favourable conditions, few predators and little competition from the native animals, increased so much that they came to be regarded as pests. Of these, the rabbit is the most notable and—perhaps surprisingly—its presence led to the introduction of yet other mammals, Mustelidae (stoats, weasels and ferrets), which it was hoped would prey upon and control the numbers of rabbits. Since they have not reduced rabbit numbers but prey upon native birds, game and poultry, the mustelids themselves are now regarded as pests.

Since many of the twenty-eight species listed in this book have been introduced in numbers during the last 75 years, ecologists in New Zealand have excellent opportunities for studying the spread of these mammals and their effects upon the vegetation and native species of animals.

Dr Wodzicki has made a thorough survey of previous work in this field and has clearly been most successful in obtaining co-operation from other departments and public bodies. While they have made it rather forbiddingly full of data, copious references to many unpublished official reports add appreciably to the value of the book, which will repay study by the reader who is really interested in New Zealand wildlife. In the survey much reliance has had to be placed on questionnaires, but the necessity for field investigations is realized.

The influence of the biotic factor on the vegetational climax is repeatedly shown. In some areas, for example, the grazing of pigs and goats (the latter introduced to provide food and also to check the spread of blackberry and other weeds and help clear the scrub and bush for sheep grazing) has caused a tussock grass community to replace primitive forest, without the help of bush-felling or fire.

The need for studies of the biology and control of mammals—if erosion is to be combated, stock maintained and the flora of National Parks preserved in its present state—is underlined again and again. To be effective, the control of mammals regarded as pests must be based on a fuller understanding of their bionomics. This predicates a long-term programme of research, directed towards the study of populations rather than individuals. While the field of such activities could be almost infinite, the author (who is in charge of the Animal Ecology Section of the New Zealand D.S.I.R.) realizes the necessity to conserve human energy as well as natural resources and is wisely concentrating initially on the mammals of greatest economic importance, beginning with the rabbit.

HARRY V. THOMPSON

Biological Standardization. By J. H. Burn, D. J. Finney and L. G. Goodwin. Pp. 440. Oxford University Press. 2nd edition. 1950. 35*s*.

This contribution to the series of Oxford Medical Publications is of obvious value to the research worker concerned with chemotherapy and pharmacology. In addition to the 225 pages covering the specialized methods for individual materials, the book includes a section of 176 pages forming a most lucid introduction to the study of the methods required for statistical analysis of the data.

Dose mortality and probits are dealt with very simply in chapter 2, in preparation for the more detailed account in the succeeding chapter by Dr Finney. This supplies a general background, which in its treatment of the fundamentals—probabilities, frequency distributions, means, standard deviations and tests for significance—presents an admirable introduction to statistical analysis for any biologist. This leads up to a section on the treatment of biological assay data, the methods being presented in simple terms for the non-mathematical reader.

The remainder of the book deals with means of standardizing some twenty-two individual products or groups of materials placed in the category of 'biological substances'. For example, methods are detailed for digitalis, strophanthus and squill, secretin and pancreozymin, and certain animal hormones. A section on vitamin D is included, illustrated with X-ray photographs, but in this edition methods for other vitamins are omitted. Certain groups of substances are assembled and the appropriate methods listed for the group. These are: antipyretics and analgesics, atropine and quinidine substitutes, local anaesthetics, substances affecting gastric secretion, and curare-like compounds. The last four chapters, by Mr L. G. Goodwin cover methods of testing the chemotherapeutic action of antihelminthic, trypanocidal, amoebacidal, leishmanicidal and antimalarial substances. In many cases illustrative data are given together with indications of the applicability of the statistical methods described earlier in the book. HELEN FERRES

An Atlas of the Chromosome Numbers in Animals. By Sajiro Makino. 2nd ed. (first American edition). Pp. xxviii + 290. Ames, Iowa: Iowa State College Press. 1951. 5.00 dollars.

The publication, now in English, of a second edition of this atlas will be of invaluable help not only to the specialist in this field, but also to the general biologists who come to need more and more such convenient and accessible sources of information of this type. 3317 species are listed (the Protozoa are not included) and the data include species name, diploid and haploid chromosome numbers (in the spermatogonia, primary and secondary spermatocytes as well as in the oogonia and oocytes), the type of heterogamety, and the bibliographic reference, arranged in a brief but admirably clear fashion. JOHN E. HARRIS

The Life of Vertebrates. By J. Z. Young. Pp. xvi + 767, with 497 figs. Oxford: Clarendon Press. 1950. 42*s*.

The appearance of a new text-book of vertebrate zoology is a rare event; when that text-book presents a new approach to the subject by an author of standing and distinction, as in the present instance, the event becomes one of importance.

Most of the better known text-books of vertebrate zoology are now old and are based on a systematic survey of the comparative anatomy of these animals. During the long interval since their appearance the teaching of Zoology has undergone considerable change. Great expansion of the subject has occurred, particularly in those branches depending on the application of the experimental, as distinct from the descriptive, method. In consequence, it has become necessary to devote a steadily increasing amount of time to comparative

physiology, experimental embryology, genetics, ecology, and so forth. Since no corresponding increase in the length of courses has occurred, the time devoted to these branches has been won largely at the expense of comparative anatomy. Yet the old text-books of comparative anatomy, which are still in use, have tended to expand in successive editions, rather than to contract, and other branches of the subject have been provided for mainly by new text-books dealing with these special fields. Hence the need for a book that would provide a synthesis of all these various aspects of vertebrate zoology has become increasingly apparent. Yet for any one person to cover such a wide field presents great difficulty; this Professor Young has succeeded in doing.

The author set out to produce a synthesis of the embryology, anatomy, physiology, biochemistry, palaeontology and ecology of all vertebrates, in fact to give a picture of the life of these animals, living and extinct, in relation to their environments. Although nearly a third of the book is devoted to a review of the mammalian orders and of their evolution, the treatment of mammalian structure, function and development had to be relegated to a future volume, in which also a survey of comparative embryology is to be included. It is very desirable that the appearance of this subsequent volume should not be long delayed, for the present work is, in a sense, unfinished without it and the section on the mammals necessarily lacks the synthetic treatment that is the principal merit of the rest of the book. Nevertheless, in my opinion, the author has achieved his aim to a remarkable extent and has produced a consistent, coherent account of the vertebrates, that gives a far more complete and balanced picture of their life than anything that has preceded it. Moreover the book is pre-eminently readable from cover to cover and is by no means a work for reference only. It would be idle to suppose that the choice of material for inclusion, or the allocation of space to the various sections, will satisfy everyone, and to us the amount of space devoted to reproduction appears unduly small, but the manner in which the material selected is presented is so good that the merits far outweigh the defects of omission.

The real test of success of a new book such as this is whether it replaces its predecessors as a recommended text for undergraduate students. No matter how useful it may be as a reference work for seniors, the extent of its influence on the subject will depend on its use as a standard undergraduate text. It is just here that this book is going to be most valuable, in the reviewer's opinion and he, at least, will have no hesitation in recommending it to his post-intermediate students.

The illustrations deserve special comment. A very large proportion of them are new and all but a very few are good. The scraper-board drawings of animals in their natural environments are excellent and very pleasing, and they are a valuable feature of the work. The quality of the anatomical and osteological drawings is good also and the two full page figures of dissections of the pigeon are especially fine. A few, such as figures 158 and 193 are not quite up to the general high standard. The publishers are to be congratulated on the size and clarity of type, and of the absence of footnotes and small type. Moreover the book is remarkably free from typographical errors, although some are inevitable in one of this magnitude. Only one feature of the production the reviewer wholeheartedly dislikes, perhaps because he attempted to read it on various train journeys; it weighs about four pounds!

F. W. ROGERS BRAMBELL

Cytology and Cell Physiology. Edited by GEOFREY H. BOURNE. Pp. xvi+524. London: Oxford University Press. 2nd ed. 1951. 50*s*.

This book was first published in 1942, but in this second edition it is nearly doubled in length and the price has increased from 20*s*. to 50*s*. In general character it is unchanged. It remains a collection of essays by collaborators, now increased in number to thirteen, who present a series of records of the state of knowledge in selected topics. There is no attempt at a synthesis of current views. Nor is there any attempt at completeness save

within the restricted fields of the separate authors. The reader who wishes to learn something of the nature of the striated border of epithelium, of cilia, or of nerve cells and their axons, will be disappointed. On the other hand, he will find very full and interesting accounts of current ideas on the cell surface, about phosphatases and their distribution or about the mitochondria and Golgi complex. In other words, he must take the book as he finds it and he will then discover that although of necessity it is uneven in writing and in content it does contain a vast amount of interesting matter, particularly on those aspects of cytology which are exciting attention at the present time. In this edition all the sections have been expanded, there are new sections dealing with modern methods in microscopy, histogenesis and tissue culture, and cytology and evolution, while the chapter on the pathological aspects of cytology has been greatly enlarged.

V. B. WIGGLESWORTH

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